ERDC TR-09-10

US Army Corps
of Engineers®
Engineer Research and
Development Center

Strategic Environmental Research and Development Program

Phytoremediation of Composition-B Derived TNT and RDX in Herbaceous Plant-vegetated and Bare Lysimeters

Elly P. H. Best, Jared C. Smith, and David B. Ringelberg

December 2009

Engineer Research and Development Center

Strategic Environmental Research
and Development Program

ERDC TR-09-10
December 2009

Phytoremediation of Composition-B Derived TNT and RDX in Herbaceous Plant-vegetated and Bare Lysimeters

Elly P. H. Best

Environmental Laboratory
U.S. Army Engineer Research and Development Center
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

Jared C. Smith

SpecPro, Inc.
3532 Manor Drive
Vicksburg, MS 39180

David B. Ringelberg

Cold Regions Research and Engineering Laboratory
U.S. Army Engineer Research and Development Center
72 Lyme Road
Hanover, NH 03755-1290

Final report

Prepared for U.S. Army Corps of Engineers
Washington, DC 20314-1000

Abstract: This report describes a study in which phytoremediation of composition-B (comp-B) derived TNT and RDX was quantified in 0.5-m *S. nutans* (Indian grass)-vegetated organic matter and nutrient-poor soil over a 92-day period. The vegetation was allowed to establish in 0.5-m-high soil cores prior to amendment with ground comp-B mixed with the same soil, and effects and fate of comp-B derived TNT and RDX were followed in plants, soil, and leachate under greenhouse conditions. Remediation in vegetated soils exceeded that in bare soils for TNT up to a comp-B level of 218 mg kg^{-1}, and for RDX up to a comp-B level of 73 mg kg^{-1}. Thus, phytoremediation can be used as an effective remediation technology in a given range of explosives contamination. The greatest annual remediation potential was 58.5 g TNT m^{-2} and 42.4 g RDX m^{-2} in vegetated soils, and 54.5 g TNT m^{-2} and 51.0 g RDX m^{-2} in unvegetated soils. Remediation was attributed to a large degree to processes other than plant uptake, including bioremediation (plant-assisted or not), complexation with plant material and soil components leading to non-extractability, and photolysis (limited to the upper soil layer). Results of a comparison between ^{15}N-based and chemical-RDX-based mass balances, with ^{15}N derived from uniformly labeled ^{15}N-RDX, indicated greater incorporation of ^{15}N than of RDX in soil and plants of vegetated units than in soil of non-vegetated units. This was attributed to the incorporation of RDX metabolites generated by the increased microbial community biomass and activity, stimulated by exuded plant compounds, and to RDX transformation within the plants themselves. Sorption coefficients for Camp Shelby soil were low, indicating considerable potential for explosives leaching. These coefficients were three times greater for TNT than RDX. Despite the considerable leaching potential derived from the measured sorption coefficients, leaching was very low compared to loss of explosives due to processes other than plant uptake. The microbial communities in the upper soil layer showed decreased biomass with increasing comp-B exposures. Community shifts were subtle if at all. However, a presumed hysteresis effect was observed in the vegetated soil at a comp-B exposure of 146 mg kg^{-1} soil.

Contents

Figures and Tables

Figures

Tables

Preface

This report was prepared by the U.S. Army Engineer Research and Development Center (ERDC), Environmental Laboratory (EL), Vicksburg, MS. The research was sponsored by the Strategic Environmental Research and Development Program (SERDP), Arlington, VA, Dr. Jeff Marqusee, Executive Director, Project Number ER1500. The principal investigator was Dr. Elly P. H. Best, Research Biologist, Environmental Risk Assessment Branch (ERAB), Environmental Processes and Engineering Division (EPED), ERDC-EL.

This report was prepared by Dr. Best, ERAB-EPED, ERDC-EL; Jared C. Smith, SpecPro Inc., Vicksburg, MS; and David B. Ringelberg, Cold Regions Research and Engineering Laboratory, ERDC. The phytoremediation capacity studies were conducted by Dr. Best and Mr. Smith. The authors gratefully acknowledge the technical assistance provided by Margaret Richmond, SpecPro, Inc., in conducting the poly lipid fatty acid determinations. The support of J. S. Furey with the stable isotopic ratio procedure interpretation and background information is also gratefully acknowledged.

The report was reviewed by Dr. Fiona Crocker, Environmental Processes Branch, EPED, ERDC-EL, and Dr. Ping Gong, SpecPro, Inc.

Nationwide Plastics, Brandon, MS, constructed the lysimeters and provided problem-solving assistance with the implementation of the lysimeter experiment. Dr. Matt Hinderleiter, The Nature Conservancy (Field Office at Camp Shelby), is gratefully acknowledged for his help with the identification of a suitable site, and the excavation and collection of surface soil at Camp Shelby, MS. Dr. J. O. Dawson, Plant Science Laboratory, University of Illinois-Urbana Champaign, is gratefully acknowledged for having his laboratory determine the soil fertility properties.

The study was conducted under the direct supervision of Dr. Richard E. Price, Chief, EPED, EL; and Dr. Beth C. Fleming, Director, ERDC-EL.

COL Gary E. Johnston was Commander and Executive Director of ERDC. Dr. James R. Houston was Director.

1 Introduction

Explosives contamination in the environment

Explosives, including 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and associated byproducts and degradation compounds may enter the environment during manufacturing, loading, assembly, and packing processes and from military activities on testing and training ranges and battlefields. Numerous explosives-contaminated sites have been identified in many countries (Hawari 2000; Pennington et al. 2001; Schroeder et al. 2003). The hazard potential, including mammalian toxicity, and mutagenic and carcinogenic features of TNT and RDX, have been reviewed by Talmage et al. (1999). Among explosives, TNT was historically the most widely used and associated with most existing soil contamination. RDX is currently the most widely used explosive. TNT and RDX are often found in the soil of the same site because they are major ingredients of Composition B (comp-B), one of the primary explosive formulations currently used in artillery projectiles and many other munitions. In soils, TNT can be strongly sorbed to clay minerals and/or organic matrices, and is, therefore, leached to a limited extent. RDX is far less strongly sorbed and has a higher potential for leaching (Dontsova et al. 2006). Because of the toxicity, mutagenicity, persistence, and longevity of explosives in the environment, substantial investments in the remediation of contaminated soil and groundwater have been made.

Phytoremediation

The need for cost-effective soil decontamination methods for large areas has increased the importance of phytoremediation as a publicly accepted strategy for in situ remediation of explosives-contaminated soil. Phytoremediation involves the use of plants to help clean the environment. It encompasses a range of processes, including phytoextraction—the use of plants to take up (accumulate) and remove contaminants from the soil—and phytostabilization—the use of both plants and soil amendments to prevent the contaminants from migrating from the source area. However, the fate and transport characteristics of explosives in vegetated soils must be understood before phytoremediation can effectively be used with confidence.

The potential of phytoremediation has been evaluated in publications (McCutcheon and Schnoor 2003; Schoenmuth and Pestemer 2004), and metabolic pathways of TNT and RDX degradation in plant tissues have been proposed (Burken et al. 2000; Van Aken et al. 2004; Best et al. 2005).

Uptake and transformation of TNT and RDX by microbes and plants

TNT is a nitroaromatic compound, characterized by an aromatic ring and three nitro groups. The strong electron-withdrawing character of the nitro group makes oxidative attack, a major mode of catalysis of aromatic compounds, increasingly difficult as the number of nitro substituents on the aromatic ring increases (Rieger and Knackmuss 1995). The same electron-withdrawing properties lead to facile reduction of the nitro group under both aerobic and anaerobic conditions. Nitroaromatic compounds are readily reduced to more reactive and, potentially, more carcinogenic and mutagenic derivatives when introduced into mammalian systems (Nishino et al. 2000). TNT is (bio)transformed by microbes under aerobic and anaerobic conditions to produce stable intermediates (amines, acetyl derivatives, azo and azoxy compounds) while maintaining its stable aromatic ring structure (Hawari et al. 1998; 1999).

TNT is taken up by both terrestrial and wetland plant species. Most TNT remains in the roots where it can be transformed into reduced TNT-degradation products, i.e., aminonitrotoluenes. Translocation to shoots is limited and no significant mineralization to CO_2 by plants has been reported. Unidentified metabolites increase over time, with low- and high-molecular composition in roots and with largely high-molecular composition in shoots (Palazzo and Leggett 1986; Hughes et al. 1997; VanderFord et al. 1997; Sens et al. 1999; Thompson et al. 1998; Bhadra et al. 1999; Best et al. 1999; Adamia et al. 2006).

RDX and HMX are cyclic nitramines, that possess N-nitro groups. RDX may undergo a change in its molecular structure, the ring collapses to produce small nitrogen-containing (N_2O, NO_2, NH_3) and small carbon-containing (HCHO, HCOOH, and CO_2) products. Both bacterial and fungal strains are known to transform RDX, and considerable mineralization into CO_2 has been reported (Fernando et al. 1990; Jones et al. 1995; Crocker et al. 2006).

RDX is taken up by both terrestrial and wetland plant species and translocated preferentially to leaf tissues where it undergoes limited transformation (Harvey et al. 1991; Larson et al. 1999; Just and Schnoor 2004; Best et al. 1999). Identified transformation products include hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX; Larson et al. 1999), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX; Van Aken et al. 2004), 4-nitro-2,4-diazabutanal, formaldehyde, nitrous oxide, and nitrite (Just and Schnoor 2004). Unidentified polar metabolites (Harvey et al. 1991) and large molecular weight adducts (Larson et al. 1999) have also been reported. Contrary to microbial degradation, no significant mineralization into CO_2 by plants has been reported (Harvey et al. 1991; Best et al. 1999; Thompson et al. 1999).

Toxicity of TNT and RDX to plants

Plants possess various strategies to detoxify organic contaminants in the environment. The 'green liver' model concept is often used to describe the fate and disposition of organic contaminants in plants since the process is similar to the metabolism in humans (Sandermann 1994). According to the green liver concept, the three steps in plant metabolism of xenobiotics are (1) transformation, (2) conjugation, and (3) sequestration. The behavior of TNT and RDX in plants complies with the green liver concept.

A few studies of the phytotoxicity of explosives have been published. Most of these, which are reviewed in Rocheleau et al. (2006), were tests of TNT. Nitroaromatic compounds such as TNT are more toxic than nitro-heterocyclic compounds such as RDX and HMX (Schnoor et al. 2006; Best et al. 2008). The published screening benchmark for TNT in soil for terrestrial plants is 30 mg kg^{-1} (Talmage et al.1999). This study is based on the Lowest Observed Effective Concentration (LOEC) of 30 mg TNT kg^{-1} for aged soil, with a No Observed Effect Concentration (NOEC) of 10 mg TNT kg^{-1} in bush bean (*Phaseolus vulgaris*; grass; Cataldo et al. 1989). The published screening benchmark for RDX in soil for terrestrial plants is 100 mg kg^{-1} (Talmage et al. 1999). This value is based on the LOEC of 100 mg RDX kg^{-1} for aged soil in cucumber (*Cucumis sativa*; Simini et al. 1995). However, a concentration of ≥ 1540 mg RDX kg^{-1} soil failed to reduce the biomass of perennial ryegrass (*Lolium perenne*) and alfalfa (*Medicago sativa*) by 20% as required for a LOEC (Best et al. 2006). A screening benchmark for HMX has not been published.

Objectives of present study

This study focused on comp-B-derived TNT and RDX. Herbaceous plants predominate the open vegetative cover of training ranges, which are areas of concern for contamination by explosives. A loamy sand from Camp Shelby, MS, was considered representative for field conditions on training ranges.

The objectives of the present study were to quantify phytoremediation, containment and leaching of solid phase comp-B-derived TNT and RDX in soil cores vegetated by herbaceous plants under environmental conditions representative for the southern part of the United States. Plant species identified as explosives-tolerant and metabolizing TNT and RDX (Best et al. 2008) were included in the tests. Effects, fate, and mass balances of comp-B derived TNT and RDX were determined in vegetated and bare soil cores over a three-month period; bioavailability was explored; microbial community composition and biomass of the upper soil layer was evaluated. Results formed the basis for estimating scaled-up phytoremediation in the field.

2 Materials and Methods

Explosives chemicals and standards

Technical grade comp-B was obtained from the Holston Army Ammunition Plant, 4509 West Stone Drive, Kingsport, TN 37660-9982. Uniformly labeled ^{15}N-RDX was purchased from Dr. G. Ampleman, Defense Research and Development Canada – Valcartier, North val-Belair, Quebec, Canada. Explosives standards were purchased as 1-mL vials, containing 1000 ± 5 μg mL^{-1} of pure substance in acetonitrile, from Ultra Scientific Analytical, NO Kingston, RI.

Experiment design

Dose-response curves for concentrations between 0 and 218.3 mg comp-B kg^{-1} soil dry weight (dry wt), constructed for the tests, were expected to result in TNT concentrations ranging from 0 to 85.1 mg kg^{-1} dry wt and RDX concentrations ranging from 0 to 131.0 mg kg^{-1} dry wt (Table 1). Comp-B is usually composed of 39% TNT, 60% RDX, and 1% wax. Among the explosives present in comp-B, TNT is the most toxic to plants and care was taken to keep the comp-B derived TNT concentration <100 mg kg^{-1} soil because the latter level was identified as lethal to tolerant plants in a previous pot experiment (Best et al. 2008).

Table 1. Core amendments with comp-B: target levels[1].

Comp-B Exposure	Amendment					
	(g core^{-1})			(mg kg^{-1} soil dry wt)		
	Comp-B	TNT	RDX	Comp-B	TNT	RDX
Control	0	0	0	0	0	0
Low	5.499	1.770	2.293	72.6	23.4	30.3
Medium	11.045	3.633	4.944	145.7	47.9	65.2
High	16.544	4.964	6.445	218.3	65.5	85.0

[1] The lysimeter units contained 75,519 g dry weight.

The lysimeters were dosed by amendment with 200 g air-dried soil containing different weights of the same comp-B stock. Lysimeters amended with 200 g air-dried soil without comp-B served as a control. All treatments were replicated three times.

Treatments for each group (bare, grasses and forbs) followed a block design, in three blocks, with each group series composed of three comp-B concentrations and a control randomized over the blocks: (three pseudo-species x three comp-B concentrations x three replicates) + (three pseudo-species x one control x three replicates). The study included a total of 36 lysimeters (Figure 1).

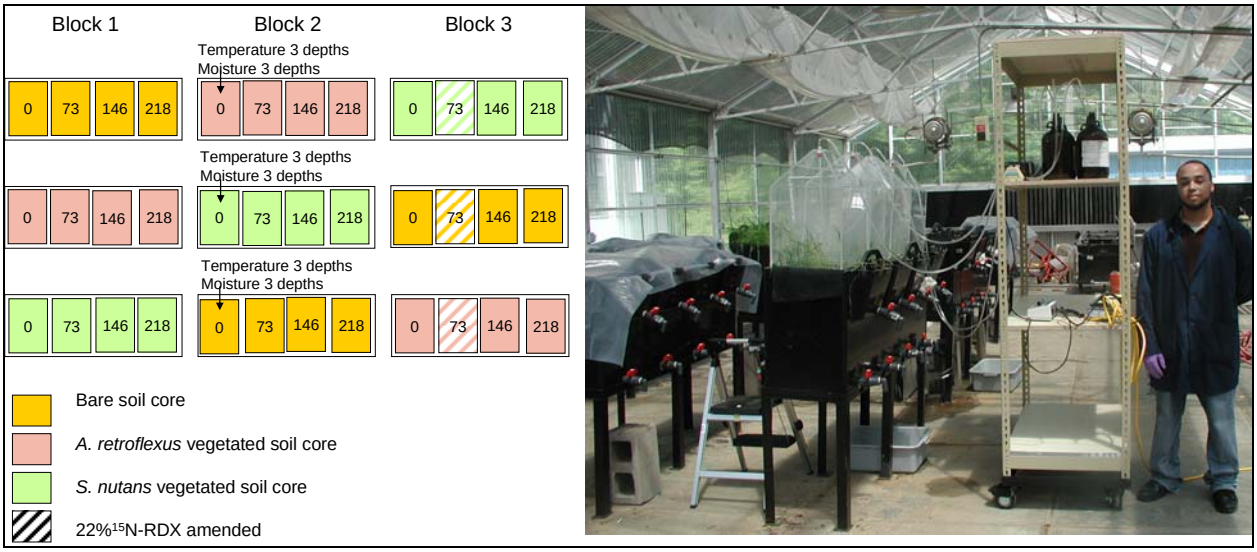

Figure 1. The lysimeter experiment followed a block design in three blocks, with each group series composed of a control and three comp-B amendments randomized over the blocks. Numbers in units represent comp-B amendments in mg kg^{-1}.

Selected lysimeters, i.e., one replicate of the units with the lowest comp-B concentration situated in Block 3, were also amended with ^{15}N-RDX for 22.0% of total RDX (0.65 g ^{15}N-RDX and 3.292 g comp-B derived RDX; three lysimeters) to track the fate of RDX in the systems and facilitate the calculation of RDX mass balances (Figure 1).

An exposure period of 92 days allowed the plants to reach maturity, and, thus, was adequate for evaluating plant persistence potential.

Lysimeters

The lysimeters were designed to allow for the collection of leachate flowing through the soil as well as runoff from the soil surface. The lysimeters were constructed from 1.905-cm-thick, high-density polyethylene that measured 0.3375 m by 0.325 m by 0.5375 m (inside length x width x height; Figure 2). Series of four lysimeters were fitted in a polyethylene envelope, placed on a stand constructed from angle iron, with a 2 degree slope for

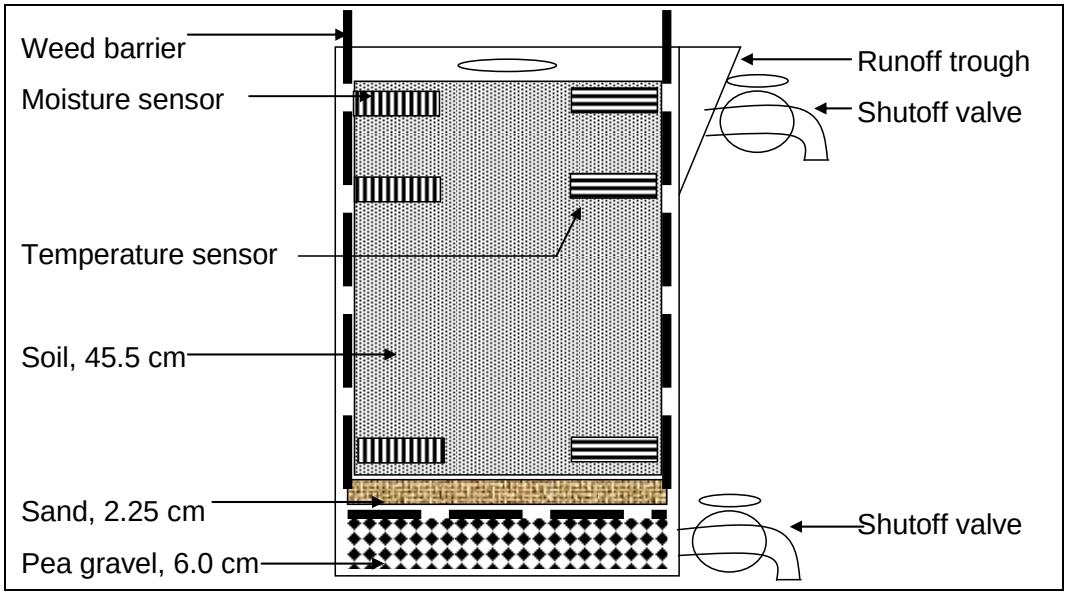

Figure 2. Cross section of a lysimeter unit.

collection of surface runoff. Sufficient room above the soil remained for a portion of the simulated rain to form a puddle and flow into the runoff trough (Figures 2 and 3). Leachate and runoff waters were collected in polyethylene pans. All tubing in the collection system was made of polyethylene.

Rainfall simulators were constructed as hoods from clear plexiglass, which were placed on top of each unit with their sides resting on the lysimeter sides. Water pressure regulators from plant spray bottles were fitted into the tops of the spray hoods to control the rainfall rate. Reservoirs containing known amounts of RO water were placed on a mobile cart. The water was transported from the reservoirs to the spray hoods through Tygon tubing using a four-channel Masterflex pump (Cole Parmer, Chicago, IL). This rainfall simulation system generated a measured quantity of simulated rainfall that flowed through the water pressure regulators of the spray hoods onto each test unit. Rainfall was administered simultaneously to four units (Figure 4).

The simulated rainfall quantity used in this study was based on 10-year average rainfall at Kosciusko, Mississippi (1472 mm (58.0 in.) per year). Rainfall at Kosciusko was used as criterium, because no long-term rainfall records of Camp Shelby were available. Kosciusko is located north of Camp Shelby; both are in Mississippi. To simulate this daily rainfall in two

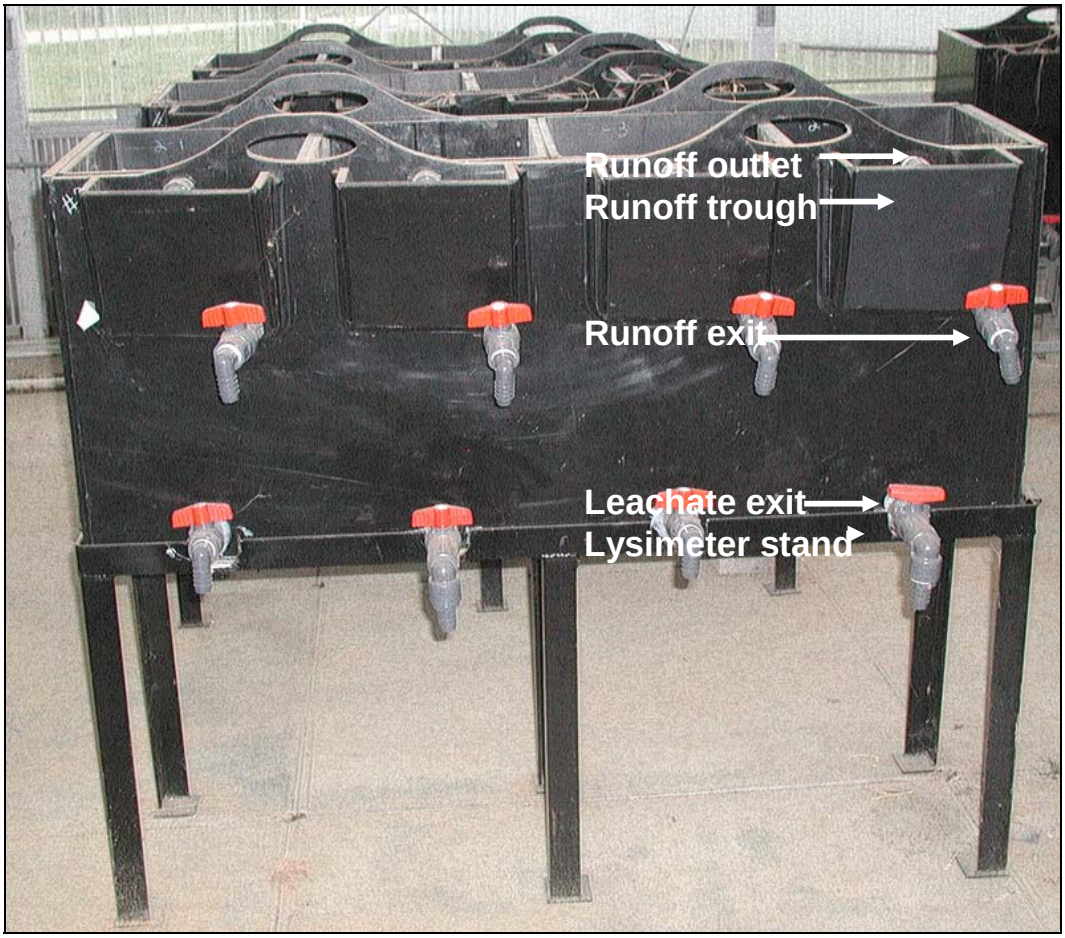

Figure 3. Empty four-unit lysimeter showing runoff and leachate collection systems.

equal portions per week, 0.556 in. or 1.66 L per event was applied to each unit. This amount was reduced in the course of the experiment to prevent the vegetation from becoming water-logged. Approximately 1 hr was required to apply the total amount of rainwater per event.

Pea gravel (6.0 cm) was placed on the bottom of the lysimeter to prevent the sediment from clogging the exit tubes during the rainfall events. A layer of weed barrier was placed on the pea gravel and fitted around the inside of the lysimeter. A 2.25-cm layer of coarse sand was placed on the weed barrier. Approximately 80.3 kg air-dried soil (75.519 kg dry soil) was placed on top of the sand layer. The soil was compacted to form a layer approximately 45.5 cm deep (Figure 2). Temperature and moisture level were monitored, using HOBO equipment (Onset Computer Corporation, Bourne, MA) in the controls of the test series in Block 2, at three depths within the soil cores: at -5 cm, within the rhizophere- if vegetated, -15 cm, just below the rhizosphere, and at -45 cm at the bottom. Monitoring was

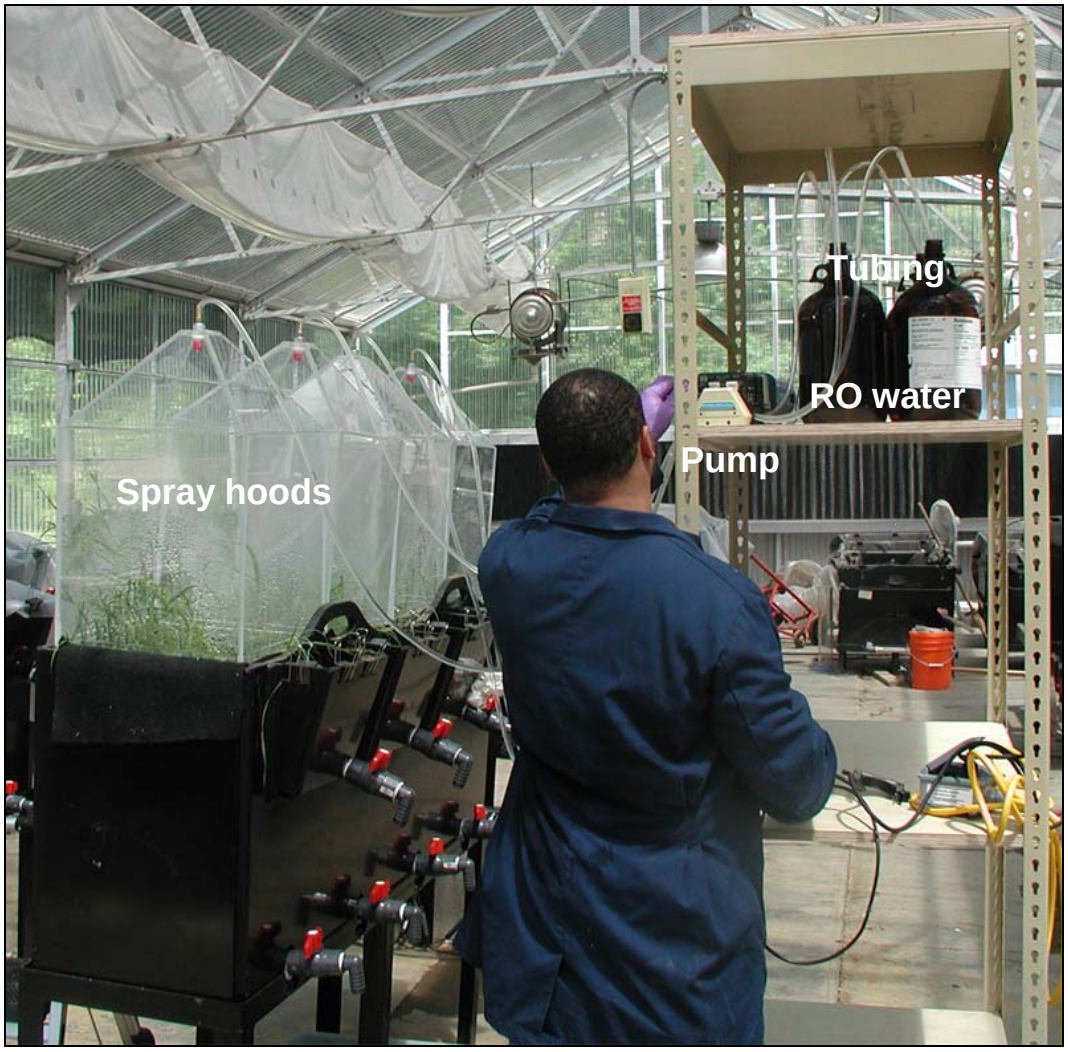

Figure 4. Lysimeter and rainfall simulation systems.

accomplished using 12-bit temperature sensors and soil moisture sensors connected to a datalogger situated in a microstation. The sensor responses were recorded at 6-hr intervals, the data transferred to a laptop using a shuttle, and 6-hr data were converted into average daily values.

Soil

Camp Shelby is located in Perry County, Mississippi, near the town of Hattiesburg. The distribution of soils in Camp Shelby was determined from the Soil Survey of Perry County, Mississippi (Daniels 1999). About 80 percent of the operational area of Camp Shelby is in the DeSoto National Forest in Perry County. Camp Shelby is a training and mobilization facility for National Guard units. About 30 different soils occur in the county, which range widely in texture, natural drainage, slope, and other

characteristics. The appearance of the soil, excavated for the experiments, agreed with the description of the McLaurin-Benndale-Smithdale association, characterized as 'dominantly nearly level to strongly sloping, well-drained loamy soils weathered from unconsolidated loamy sediments.' It agreed most with the McLaurin characteristics, i.e., surface layer dark grayish brown fine sandy loam; subsurface layer yellowish brown, fine, sandy loam; subsoil upper part-yellowish red sandy loam with red mottles; lower part—red sandy loam; well-drained. Soil was collected from the primary soil site in the northern part of Camp Shelby. This site was selected because it was sparsely vegetated by pine trees and herbaceous vegetation, regularly used for surface soil excavation, and easily accessible (Figure 5). Vegetation and surficial detritus were removed, and surface soil to a depth of 0.3 m was excavated using a bulldozer (Figure 6), and transferred to the back of a truck. The soil was transported to the Environmental Laboratory, Vicksburg, MS, for further processing.

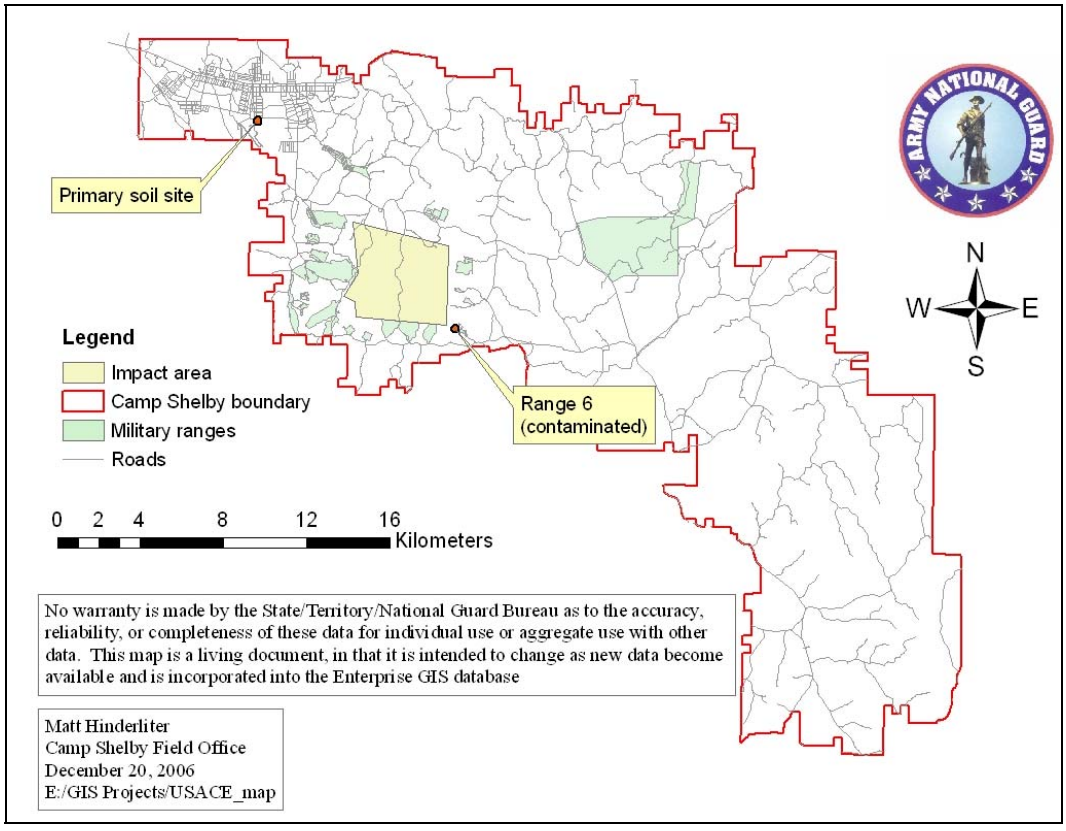

Figure 5. Location of soil excavation site within Camp Shelby, MS.

Figure 6. Collecting soil from Camp Shelby, MS.

The soil was air-dried, turned, and mixed using a skipsteer in a hangar open to the air until a soil moisture content of 6% was reached. The soil was subsequently freed from stones, transferred into 190-L barrels, and transported to the greenhouse on the Environmental Laboratory grounds until the experiment was conducted.

For the tests, the lysimeters were amended with 5.499, 11.045, and 16.544 g comp-B aliquots per core as follows. Pre-weighed amounts of comp-B were ground in a porcelain mortar into a fine powder, and added to pre-weighed amounts of mortar-ground (to pass a 2-mm sieve) soil to total weights of 200 g air-dry amendment. These mixtures were mixed overnight on a modular roller (Wheaton Science, Millville, NJ) in opaque, glass, 350-mL IChem jars. The amendments were stored in darkness at room temperature until use. Handling and processing of comp-B were shielded from light as much as possible. The amendments were applied to the lysimeters after the vegetation was established: 2 weeks after initiating the watering regime of the bare and grass-vegetated units, and 4 weeks later of the forb-vegetated units.

For the ^{15}N tests, the amendments already containing 5.499 g comp-B (2.293 g RDX) were sprayed with an additional 0.65-g ^{15}N-RDX in 65 mL methanol. Thus, the RDX levels in these lysimeter units were targeted at 22.0% ^{15}N-enrichment. After spraying, the soils were mixed with a

stainless steel scoop, and placed in a vented fume hood without illumination overnight to allow the methanol to evaporate prior to contacting the plants.

Plant materials

Two plant species previously identified as tentative candidates for inclusion in the explosives phytoremediation experiment (Best et al. 2008) were purchased: Redroot pigweed (*Amaranthus retroflexus)* was purchased from Azlin seed service, Leland, Mississippi, and Indiangrass (*Sorghastrum nutans*) from the Granite Seed Company, Lehi, Utah.

Exposures

For each unit, a weight equivalent to 2,675 seeds (6.41 g *A. retroflexus* and 44.18 g *S. nutans*) was placed on top of air-dry soil cores equivalent to 75.519 kg soil-dry wt, contained in 58.9-L lysimeters. The grass seeds were seeded directly after weighing on 5 April 2008, and the forb seeds were seeded after overnight soaking in 0.5 mg L^{-1} gibberellic acid to enable rapid, synchronized germination. The lysimeters were covered with transparent, plastic lids, and sprayed with Reverse Osmosis (RO) water immediately after placing the test seeds on the soils, and, subsequently, every day as needed until seedlings were visible.

The grass germinated readily and had established a vegetative cover on 19 May. Subsequently, the grass-vegetated units were watered twice a week with RO water to stimulate growth of the vegetation. The bare units were watered also. On 29 May, the bare and grass-vegetated units were saturated with RO water by repeated irrigation of the top. After complete saturation occurred, as evident from standing water remaining visible on top of the soil and vegetation mass, the excess water was drained from each lysimeter to a soil moisture level of 36%. From 29 May onwards, these units were subjected to a simulated rainfall regime as described earlier, which kept the soil at a moisture level close to field capacity (approximately 36%; field capacity was 38%). A moisture level at field capacity allows maximum mobility of contaminants in soil solution. The grass-vegetated and bare units were amended on 2 June by spreading the appropriate comp-B/soil mixtures as homogeneously as possible over each core surface. After each rainfall event, 100-mL leachate samples were collected if possible. In several cases sample intervals and size became irregular, because selected lysimeters became temporarily clogged and/or

leaked until repaired. In all cases sample volume was recorded and explosives concentrations were determined. The water balances are presented in Table 2. The sample volumes comprised 20 to 31 % of the rainfall volumes and were greatly exceeded by evapotranspiration. Evapotranspiration rates were 1.9 to 2.4 L m^{-2} d^{-1}, similar to those measured in the previous pot experiment (Best et al. 2008).

Table 2. Water balances of soil-plant systems. Measured values, means, and standard deviations are shown (N=3).

Comp-B Exposure	Block	Initial Water (mL $unit^{-1}$)	Rainfall Water (mL $unit^{-1}$)	Sampled Water		Final Water (mL $unit^{-1}$)	Evapotranspiration	
				(mL $unit^{-1}$)	(% rainfall)		(% rainfall)	(mL m^{-2} d^{-1})
Bare								
73 mg kg^{-1}	1	36974	28600	5030	17.6	36974	82.4	2310
73 mg kg^{-1}	2	36974	28600	8170	28.6	36974	71.4	2003
73 mg kg^{-1}	3	36974	34600	5505	15.9	36974	84.1	2852
Mean ± SD		3694 ± 0	30600 ± 3464	6235 ± 1693	20.7 ± 6.9	3694 ± 0	79.3 ± 6.9	2388 ± 430
146 mg kg^{-1}	1	36974	28600	5885	20.6	36974	79.4	2227
146 mg kg^{-1}	2	36974	28600	7450	26.0	36974	74.0	2073
146 mg kg^{-1}	3	36974	34600	4104	11.9	36974	88.1	2989
Mean ± SD		3694 ± 0	30600 ± 3464	5813 ± 1674	19.5 ± 7.2	3694 ± 0	80.5 ± 7.2	2430 ± 491
218 mg kg^{-1}	1	36974	28600	7050	24.7	36974	75.3	2112
218 mg kg^{-1}	2	36974	28600	14350	50.2	36974	49.8	1397
218 mg kg^{-1}	3	36974	28600	7725	27.0	36974	73.0	2046
Mean ± SD		3694 ± 0	28600 ± 0	9708 ± 4034	33.9 ± 14.1	3694 ± 0	66.1 ± 14.1	1852 ± 395
S. nutans Vegetated								
73 mg kg^{-1}	1	36974	27100	7520	27.7	36974	72.3	1919
73 mg kg^{-1}	2	36974	32000	5173	16.2	36974	83.8	2630
73 mg kg^{-1}	3	36974	30400	14660	48.2	36974	51.8	1543
Mean ± SD		3694 ± 0	29833 ± 2499	9118 ± 4941	30.7 ± 16.2	3694 ± 0	69.3 ± 16.2	2031 ± 552
146 mg kg^{-1}	1	36974	27100	6920	25.5	36974	74.5	1978
146 mg kg^{-1}	2	36974	27100	10680	39.4	36974	60.6	1609
146 mg kg^{-1}	3	36974	27100	5170	19.1	36974	80.9	2150
Mean ± SD		3694 ± 0	27100 ± 0	7590 ± 2815	28.0 ± 10.4	3694 ± 0	72.0 ± 10.4	1912 ± 276
218 mg kg^{-1}	1	36974	27100	5970	22.0	36974	78.0	2071
218 mg kg^{-1}	2	36974	28100	11600	41.3	36974	58.7	1617
218 mg kg^{-1}	3	36974	26000	2313	8.9	36974	91.1	2322
Mean ± SD		3694 ± 0	27067 ± 1050	6628 ± 4678	24.1 ± 16.3	3694 ± 0	75.9 ± 16.3	2003 ± 357

The forbs exhibited poor germination initially. These units were reseeded on 24 May with similar results. The units were reseeded again on 30 May and seeds were covered by a thin layer of soil to enable more intensive contact between seeds and soil. Forb germination remained poor and the seedlings barely grew. However, the forb-vegetated units were amended on 30 June with the appropriate comp-B/soil mixtures in anticipation of an increase in growth.

The lysimeter study was conducted in a greenhouse of the Environmental Laboratory, Vicksburg, MS, to enable administering a known rainfall regime, estimating the water balances of the lysimeters, and avoiding contamination of the environment. The exposures lasted from 2 June to 2 September 2008 (92 days; Figure 4).

Sampling

The following parameters were determined:

- In plants as a basis for the evaluation of plant response:
  - Biomass characteristics (above- and belowground biomass, root length and root surface area) at the end of the exposure period
  - Concentrations of explosives and metabolites at the end of the cultivation period
  - $\delta^{15}N$ at the end of the exposure period in ^{15}N-amended units and in non-^{15}N amended units with the same RDX levels
- In leachates as a basis for the evaluation of explosives loss with leaching versus containment:
  - Volume, twice per week
  - Concentrations of explosives and metabolites, twice per week
  - Bioavailability of explosives via solid phase micro extraction (SPME), monthly
- In soils as a basis for the evaluation of soil-based explosives remediation:
  - Concentrations of explosives compounds and metabolites initially and at the end of the exposure period
  - $\delta^{15}N$ in the amendments and at the end of the exposure period in ^{15}N-amended units and in non-^{15}N amended units with the same RDX levels
  - Microbial community biomass and relative composition using polar lipid fatty acid methyl esters (PLFAME) as a semi-quantitative measure

The *S. nutans* plants were harvested (three 10-cm x 10-cm squares per unit) and divided into above- and belowground plant portions using stainless steel scissors. The plant tissues were washed in RO water to remove dust and soil particles, blotted as dry as possible, and weighed. After collecting, washing, blotting, and weighing were completed, plant tissues were placed in plastic Ziploc bags and kept refrigerated. Subsamples were used directly for determination of dry weight, root length, and surface area, but after storage at −80 °C for determination of explosives compounds. Almost no *A. retroflexus* plants survived, and, therefore the *A. retroflexus* seeded units were neither sampled nor analyzed.

Small aliquots of the soil amendments were stored in darkness at room temperature until analysis. The exposed soil cores were sampled in three layers: a top layer (surface to -7.5 cm), a mid layer (-7.5 to -15.0 cm), and a bottom layer (-15 to -45.5 cm) using an auger with an ID of 5.3 cm. All layer samples were homogenized using a stainless steel scoop, placed in plastic Ziploc bags, and refrigerated until further processing.

Water samples (100-mL) were collected twice per week after each simulated rainfall, the sampled volumes were recorded, and the samples refrigerated until further processing.

Sample processing and analyses

Plant biomass characteristics

Dry weight was determined by drying the fresh material in a forced-air oven to constant weight (105 °C).

Root systems were characterized by determining total length (m) and surface area (cm^2) using a WinRHIZO system (WinRHIZO Pro LA2400; Regent Instruments Inc., Quebec, Canada). For these determinations, subsamples of the washed root systems of a known weight were spread as homogeneously as possible in a translucent tray, black and white images were collected using an Epson LA2400 scanner equipped with a backlighting source (EPSON Expression 10000XL 1.0 TWAIN source), and images were analyzed using the WinRHIZO Pro software package. Because the surface area:weight ratio differed considerably among plant species, the weights of the root subsamples subjected to the scanning procedure were selected to ensure a standard deviation of 2% for three subsamples of the same root system (range 0.2-0.4 g fresh wt).

Explosives in plants, leachates, and soil

Plant tissue explosives and degradation compounds were determined using a modification of method 8330 for soils (U.S. Environmental Protection Agency (USEPA) 2006) described below. Plant extracts were prepared from freshly ground materials. Three replicate samples of each treatment were extracted. Plants were clipped into small pieces and mixed. Subsamples for extraction were homogenized by grinding them in liquid nitrogen. 2-g fresh wt portions were spiked with 1,3-dinitrobenzene (13DNB) as internal standard for recovery (50 uL of a 1 mg mL^{-1} solution), and extracted in 5-mL acetonitrile by an 18-hr sonication in a water-cooled bath at 15 ^{o}C. The extracts were freed from particles by centrifugation for 10 min at 2,000 g. 2-mL aliquots of the supernatants were cleaned over a 0.5-g Florisil Solid Phase Extraction (SPE) column, concentrated 10x by evaporation under a stream of N_2 at 35 ^{o}C. The final sample volume was adjusted to 1.5 mL with 1:1 acetonitrile: Millipore-filtered RO water. The samples were freed from remaining particles by cleanup over a 0.45-µm polytetrafluroethylene (PTFE) disk, and analyzed using High Performance Liquid Chromatography (HPLC). The extracts of the samples in which the highest explosives levels were expected were first screened for the presence of all compounds listed by USEPA Method 8330 (USEPA 2006), for the RDX degradation compounds with one, two, and three N-groups substituted (MNX, DNX, and TNX), and for 13DNB. After identifying the explosives' parent compounds and metabolites in these extracts, only the relevant compounds were determined in all other extracts. The relevant compounds in plants were usually: 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2ADNT), 4-amino-2,6-dinitrotoluene (4ADNT), hexahydro-1,3,5-trinitro-1,3,5 triazine (RDX), octahydro-1, 3,5,7-tetranitro- 1,3,5,7- tetrazocine (HMX), and 1,3 dinitrobenzene (13DNB) as internal standard.

Leachate samples (100 mL) were concentrated on 500-mg Sep-Pak Porapak RDX cartridges (No. 47220, Waters; Jenkins et al. 1995), eluted with acetonitrile, concentrated by a factor of approximately 100 x by evaporation under a stream of N_2 at 35 ^{o}C. No internal standard was used in this analysis. The relevant compounds in water were usually: TNT, 2ADNT, 4ADNT, 24DANT, RDX, MNX, DNX, and HMX.

Soil extracts were prepared from air-dry material. Three replicates of each amendment level and one replicate of each exposed soil core layer were extracted and analyzed for explosives residues. 2-g air-dry weight was

extracted in 5-mL acetonitrile by 18-hr sonication at 15 ^{o}C, cleanup over a Florisil column, 10x concentration, and cleanup over a PTFE disk. The relevant compounds in soil were usually: TNT, 2ADNT, 4ADNT, RDX, MNX, HMX, and 13DNB.

The method detection level (MDL) in mg kg^{-1} DW for several target explosives compounds, spiked on plants and soil directly before extraction, varied with compound.

- In freshly ground plant tissues:
  - MDL: TNT 0.081, 2ADNT 0.103, 4ADNT 0.161, 4NT 0.314, RDX 0.142, HMX 0.110 mg kg^{-1} DW
- In air-dry ground soil:
  - MDL: TNT 0.1684, 2ADNT 0. 3043, 4ADNT 0.1225, RDX 0.3122, HMX 0.1913 mg kg^{-1} DW
- Recovery of 1,3 DNB was usually 95%.
- The detection level in μg L^{-1} TNT and RDX in leachate was > 0.1 μg L^{-1} TNT and RDX.

Stable isotope analysis

The δ^{15}N-isotopic ratios in plants were determined in freeze-dried subsamples of the liquid N_2-ground plant materials. Plant materials exposed for 28, 63, and 92 days were analyzed. The δ^{15}N-isotopic ratios were determined in freeze-dried subsamples of the amendments, and of the three soil layers after 92-d exposure. All analyses were done in triplicate, except when not enough material was available. In addition to δ^{15}N-isotopic ratios, δ^{13}C-isotopic ratios, carbon, and nitrogen contents were also analyzed using a Thermo-Finnigan Delta-plus Advantage gas isotope-ratio mass spectrometer, interfaced with a Costech Analytical ECS4010 elemental analyzer at the Colorado Plateau Stable Isotope Laboratory in Flagstaff, CO. The following isotope calibration standards were used: for δ^{13}C, IAEA CH6, IAEA CH7; for δ^{15}N IAEA N1, IAEA N2, IAEA 305A, IAEA 310A, IAEA 310B, IAEA 305B, IAEA 311. Elemental calibration standards included: acetanilide, BBOT, cystine, methionine, sulfanilamide, cyclohexanone, and nicotinamide. NIST pine needles served as a secondary check standard. The δ^{15}N isotopic ratios, measured in samples, are reported in units per mil (‰, defined to be a relative change of 10^{-3}) calculated relative to the ^{15}N isotopic fraction in the atmospheric nitrogen defined as Vienna standard air (VAIR) reference maintained by the IAEA.

The typical atmospheric nitrogen value of 0.3663×10^{-3} (Platzner et al. 1999) can be used as the ^{15}N fraction of VAIR for calculation.

For mass balance using stable isotope ratios, the relative measured δ must be used to calculate the isotope fraction F using the defined reference value. Since

$$\delta^{15}N \text{ ‰} \frac{F - 0.3663 \times 10^{-3}}{0.3663 \times 10^{-3}} \quad (1)$$

then

$$F = \delta^{15}N \times 0.3663 \times 10^{-3} + 0.3663 \times 10^{-3} \quad (2)$$

Along with the total mass of nitrogen in a sample (N), this fraction is used to calculate the total isotope mass in the sample (^{15}N) via

$$^{15}N = F \times N$$

Bioavailability estimates of TNT and RDX via SPME

SPMEs have been used successfully to predict bioavailability and toxicity of organic compounds, including TNT in soil, sediment, and water (Mayer et al. 2000; Wells and Lanno 2000; Conder et al. 2003; Conder and La Point 2005). SPMEs are thin silica fibers coated with a microlayer of organic polymer. When exposed to environmental matrices, the polymer sorbs organic compounds to concentrations several orders of magnitude higher than that of the surrounding matrix. As long as the fiber-coating volume to sample volume ratio remains small, the SPME coating does not exhaustively extract compounds and only sorbs dissolved or weakly dissociable molecules from solution. In sediments and wet soils, the SPME approach may yield a more accurate surrogate measurement of the partitioning process between organisms and matrix compared to sediments/soil concentrations normalized by organic carbon; thus, SPME may be representative of bioavailability. The objectives of the present SPME application were to explore the ability of SPMEs to predict the bioavailabilities of TNT and RDX to plants.

For the present study, the only fiber known to adsorb both TNT and RDX (Supelco 1997) was used. The SPME (65-µm polydimethylsiloxane coated with divinylbenzene, PDMS/DVB, on a polyacrylate core) was purchased

in bulk (without syringe applicator) from Supelco (Bellefonte, PA). Fiber was cut into 1.00-cm pieces using a double-bladed, stainless-steel razor blade. Each 1-cm fiber contained 0.418 μL of adsorption phase. Two 1-cm SPMEs (0.836 μL) were inserted halfway through pierced, stapled, Teflon-coated silicone disks (septa for glass vials; diameter, 10 mm; thickness, 1 mm). The SPME disks were then rinsed with 50:50 HLPC-grade acetonitrile:ultrapure water, rinsed with ultrapure water, and allowed to dry at room temperature. The SPME disks allowed the SPMEs to be handled and exposed to the explosives in the solutions by immersion using forceps without damage or loss of the SPME. The staple in each disk allowed both disk and fiber to be recovered easily with a magnet. Fiber uptake of TNT and RDX is independent of the disk; this was verified by measurement.

Once per month, the bioavailability of TNT and RDX was determined in the leachates using SPMEs. The SPME disks were exposed to 10-mL leachate volumes contained in 15-mL glass vials, capped, and strongly agitated on a shaker (Model Innova 2050; New Brunswick Scientific, NJ) for 24 hr in darkness at room temperature. An exposure time of 24 hr was sufficient for TNT and RDX to reach steady state concentrations in SPME's (Table A1). At termination of the exposures, each SPME disk was retrieved and removed from its leachate-containing vial using stainless steel forceps, adhering solution removed by touching a paper tissue, and placed into an HPLC autosampler vial containing 1.2 mL of 50:50 HPLC-grade acetonitrile:ultrapure water for 90 min to desorb compounds from the fiber (Figure 7). The SPME extracts were analyzed using HPLC as described previously. The SPME data are presented as concentrations (mg compound adsorbed by PDMS/DVB divided by volume of PDMS/DVB), similar to those presented in the study by Conder and La Point (2005) from which this procedure was derived. The SPME method detection limits were 0.1 mg mL^{-1} for TNT and 1 mg L^{-1} for RDX, with linear responses between 0.1 and 10 mg L^{-1} for TNT and between 1 and 5 mg L^{-1} for RDX (Figure A1). The SPME disks exposed for 24 hr to the leachates and desorbed in 50:50 acetonitrile:water were expected to provide a reasonable indication of TNT and RDX bioavailability for TNT and RDX in the leachates, since the aqueous TNT concentrations were >0.1 and <10 mg L^{-1} and aqueous RDX >1.0 and <3.5 mg L^{-1}.

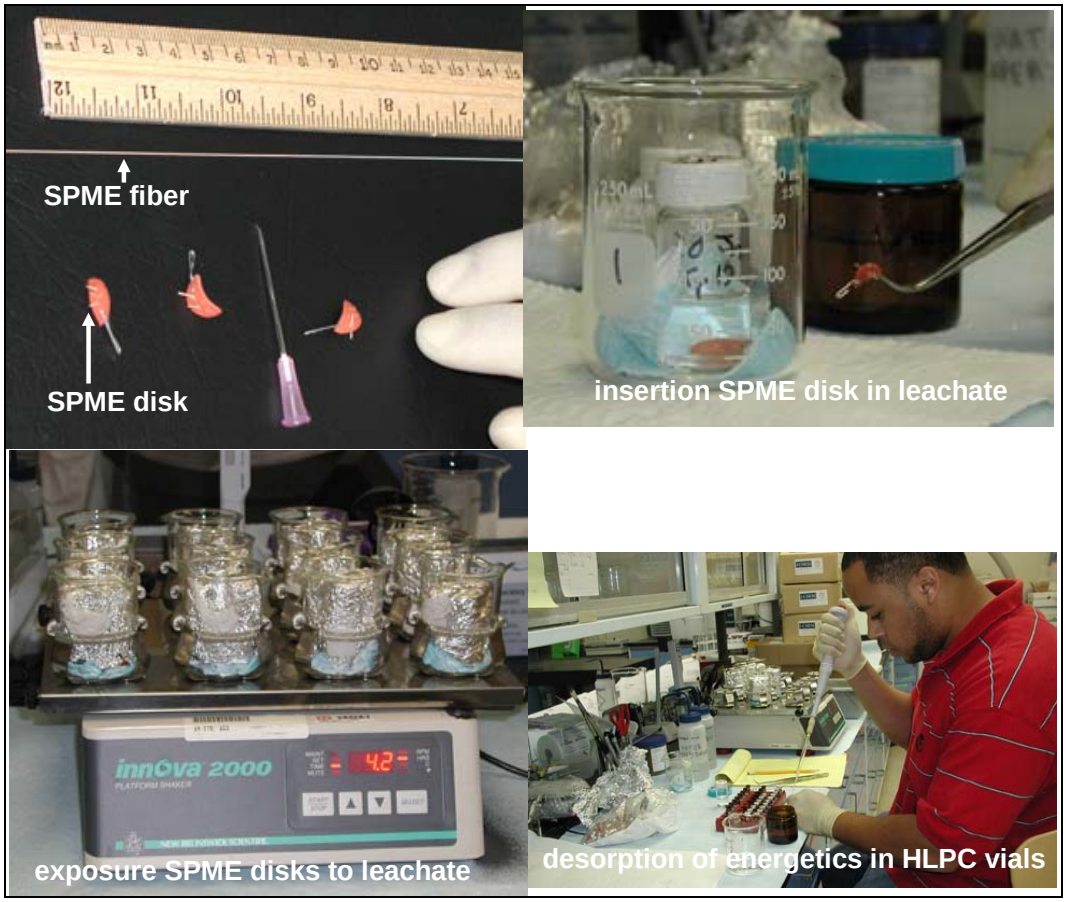

Figure 7. Handling of SPMEs to evaluate bioavailability of explosives in soil leachates.

Polar lipid fatty acid methyl ester (PLFAME) analysis

The PLFAME analysis has been shown to give an accurate estimate of the microbial cells present in soil because it does not require the cultivation of fastidious environmental microorganisms. 1 pmole of PLFAME is assumed to be equivalent to 2.5 x 10^7 microbial cells (Pinkart et al. 2002). Lipids were extracted from the initial soil and from the top layers of the soil cores included in the lysimeter experiment using a modified Bligh-Dyer (Bligh and Dyer 1959) extraction procedure (Pinkart et al. 2002). A 2-g soil fresh weight portion was extracted at room temperature by placing it in 6 ml of a mixture of dichloromethane: methanol: water (1:2:0.8, v:v:v) for 3 hr and then in an ultrasonic bath at 10 ^{o}C for 2 min. Samples were allowed to stand an additional 4 hr at room temperature before the liquid phases were separated by the addition of equal volumes of dichloromethane and distilled water. Samples were centrifuged for 15 min at 2000 rpm, and the dichloromethane phase was removed with a pipette and placed in a clean test tube. The dichloromethane phase, containing all the extractable lipids, was dried under a nitrogen stream at 37 ^{o}C. Amino-propyl solid phase

extraction columns (SPE; Agilent, ACCUBOND II Amino Cartridge, #188-1050) were used to separate the total lipid into neutral, glyco- and polar lipid fractions. Phospholipid fatty acid methyl esters (from the polar lipid fraction) were prepared for gas chromatography/ mass spectrometry (GC/MS) by mild alkaline methanolic transesterification. The resulting phospholipid fatty acid methyl esters were dissolved in hexane containing methyl nonadecanoate (C19:0; 50 pmol μL^{-1}) as an internal standard and analyzed using a gas chromatograph equipped with a 60-m x 0.25-mm (i.d.) DB-5MS capillary column (0.1-µm film thickness, J&W Scientific, Folsom, CA) and a flame ionization detector. Peak identities were confirmed using a gas chromatograph-mass selective detector (Hewlett Packard GC6890-5973) with electron impact ionization at 70 eV. Areas under the peaks were converted to concentrations based on a comparison to the internal standard. The total area of all PLFAME peaks in a GC trace were summed to provide a measure of the total soil microbial community biomass, and these data were normalized to the gram weight extracted. For determining PLFAME microbial community profiles, the raw chromatographic data were exported to EXCEL, and retention times were adjusted and rounded to the nearest 0.05 min. Once peaks were aligned, a principal component and means comparison were performed. The data were subjected to a Fisher's least significance difference (LSD) to determine significance to the bare and the vegetated means. The principal component analysis was used because no a priori hypothesis was tested. It attempts to minimize the sum of squares of any two clusters found at each step of an algorithm. It was used to determine if a significant relationship existed between sets of data for the bare and the vegetated units. For this analysis the profiles were arcsine square root transformed.

Other soil analyses

Other chemical and physical characteristics of the unamended soil were also determined in triplicate, except for particle distribution, where one replicate was analyzed. The results of these analyses are presented in Table 3.

- Kjeldahl nitrogen was measured in potassium persulfate digests using standard procedures (Bremner and Mulvaney 1982). Nitrate and ammonium nitrogen were determined according to Keeney and Nelson (1982).

Table 3. Properties of clean[1] Camp Shelby soil prior to the amendments and tests. Mean values and standard deviations are shown (N=3).

Property	Level
Nutrients and Ions	
Kjeldahl-nitrogen (mg kg^{-1} dry wt)	266 ± 19
Nitrate-nitrogen (mg kg^{-1} dry wt)	5 ± 0
Ammonium-nitrogen (mg kg^{-1} dry wt)	52 ± 4
Total phosphorus (mg kg^{-1} dry wt)	9.0 ± 2.4
Potassium (mg kg^{-1} dry wt) [%saturation]	53.0 ± 5.2 [2.3]
Calcium (mg kg^{-1} dry wt) [%saturation]	688.0 ± 48.6 [58.4]
Magnesium (mg kg^{-1} dry wt) [%saturation]	136.0 ± 19.3 [19.3]
Hydrogen [%saturation]	[20]
Other	
pH water	5.79 ± 0.02
Organic matter (% dry wt)	5.50 ± 0.17
Dry weight (% fresh wt)	98.65 ± 0.05
Bulk density (g dry wt mL^{-1})	1.98 ± 0.15
Cation exchange capacity (meq 100 g^{-1} dry wt)	4.85 ± 0.16
Sand (% dry wt)	89
Silt (% dry wt)	6
Clay (% dry wt)	5
Adsorption coefficient TNT (K_d; L kg^{-1} dry wt)	1.587
Adsorption coefficient RDX (K_d; L kg^{-1} dry wt)	0.569

[1] This soil was not subjected to explosives and organics analyses since no prior history of exposure existed.

- Phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were determined by atomic absorption spectrophotometry in malic acid extracts; phosphorus according to Frank et al. (1998), and K and other basic cations according to Warncke and Brown (1998). The cation CEC was determined mathematically from the values for P, K, Ca, Mg, and pH. pH, nutrients, basic cations and cation exchange capacity (CEC) were determined according to Recommended Chemical Soil Test Procedures for the North Central Region of the United States.
- pH. pH was determined in a 1:1 soil:water buffer solution, consisting of 37 g KCl, 215.25 g KOH, 10 g nitrophenol, and 7.5 g boric acid per L of water (Watson and Brown 1998).
- Organic matter. Concentrations of organic matter were determined by loss on ignition at 550 °C, and bulk density volumetrically (Allen et al. 1974).

- Moisture. The moisture content was determined by drying at 105 °C in a forced-air oven until constant weight.
- Particle size distribution was determined using the hydrometer method of Day (1956), modified by Patrick (1958). Particles were separated into size fractions of >50 μm (sand), 50-2 μm (silt), and 2 μm (clay) by suspension of 40 g dry material in 1 L of s dispersing solution (sodium metaphosphate at pH 8.3). An ASTM hydrometer (152H) placed in the suspension was read at specified sedimentation times for the various size fractions based on Stokes' equation. Results were reported as percentage of sand, silt, and clay.
- Sorption capacity for TNT and RDX. The sorption capacity of the soil was determined following Brannon et al. (2002). Approximately 1 kg dry Camp Shelby soil was ground in a mortar, sieved through a 2-mm mesh sieve, and homogenized overnight on a Wheaton roller. The tests were conducted in triplicate in 25-mL centrifuge tubes containing weighed 4-g soil aliquots. Tests were spiked with 16 mL of distilled deionized water containing either TNT or RDX at concentrations of 1, 2.5, 5, 7.5, and 10 mg L^{-1}. Initial aqueous explosives concentrations were verified using HPLC analysis. Solutions of TNT and RDX were 1% radiolabeled and 99% unlabeled. Uniformly ring-labeled TNT ([(ring-^{14}C)TNT] (New England Nuclear Research Products, Boston, MA) having a specific activity of 6.56 mCi/mmol and a chemical purity >98% was used for TNT studies. Uniformly ring-labeled RDX ([(ring-^{14}C)RDX] (New England Nuclear Research Products, Boston, MA) having a specific activity of 48.00 mCi/mmol and a chemical purity >98% was used for RDX studies. Following addition of the test solutions, the centrifuge tubes were sealed, shaken for 24 hr on a horizontal shaker at 180 excursions per min to reach equilibrium (Dontsova et al. 2006), then centrifuged for 15 min at 74000 RCF, and sampled removing 1 mL of the aqueous phase. The samples were counted in a Packard Tricarb 2500 Liquid Scintillation Analyzer (Packard Instruments Inc., Meriden, CT). The soil-adsorbed explosives were calculated by subtracting the final aqueous explosives content of the tube from initial, and dividing by the weight of the soil aliquot tested. The K_d's were determined from the isotherms and expressed as L kg^{-1}.

Data analysis

Statistical analyses were conducted with the software STATGRAPHICS Plus 5 for Professionals package (Manugistics Inc., Rockville, MD). Normal distribution of the data was tested using the Shapiro-Wilk's test.

Analysis of variance (ANOVA) was conducted and expanded in several cases with a multiple range test using the Fisher's least significant difference procedure. The p-value in the ANOVA is a measure of the significance of the analysis. A p value of ≤ 0.05 indicates a 95-percent confidence level.

Linear regression analyses were conducted using the least squares method. Non-linear equations were fitted with the polynomial regression module using the least squares method. The p-value of ≤ 0.05 of the regression model indicates a 95-percent confidence level. The R^2-value of the regression model indicates the proportion of the variance explained by the model. Regression models explaining at least 50 percent of the variability in the data set, i.e., $R^2 \geq 0.50$, were considered as meaningful.

3 Results

Soil properties, explosives adsorption characteristics, and extractable explosives in the amendments

Camp Shelby soil showed characteristics typical for a sandy loam, including a high sand content, and low silt and clay contents. The low organic matter content concomitant with the low levels of nitrogen, phosphorus, and potassium contributed to the low nutrient potential for vegetative growth (Table 3), with nitrogen most likely limiting growth. Low plant-nutritive soil characteristics are typical for military training lands, which are usually located in areas with poor soils unsuitable for agriculture.

Both the TNT and RDX isotherms fitted to the soil sorption data were linear (Figure 8). The K_d values determined from the isotherms of Camp Shelby soil were 1.59 L kg^{-1} for TNT and 0.57 L kg^{-1} for RDX. This indicated that TNT sorbed about three times as strongly to Camp Shelby soil as RDX, making TNT less amenable for leaching than RDX. The K_ds were low, and typical for a soil with a low CEC of 4.85 meq 100 g^{-1} dry wt and low total organic carbon (TOC) content of 2.2% (derived from the measured organic matter content, Table 3).

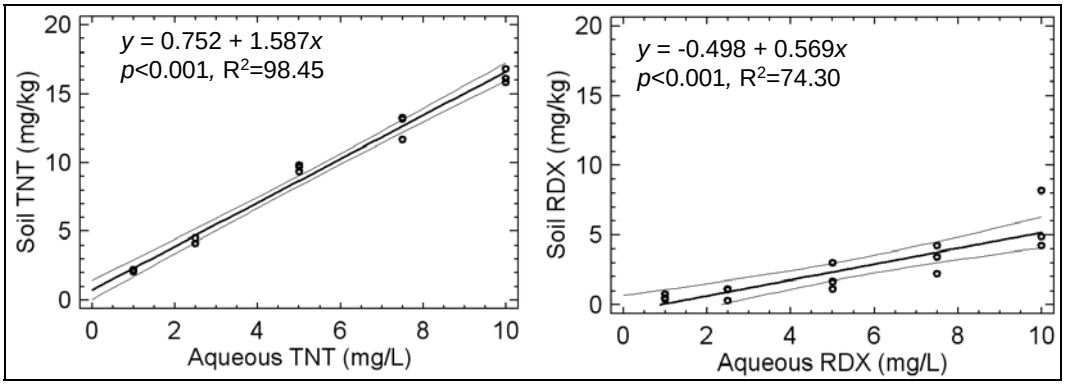

Figure 8. Adsorption isotherms for TNT and RDX with Camp Shelby soil. Aqueous explosives measured solution concentration. Soil explosives calculated soil concentration. Regression lines and 95% confidence limits indicated *y* = soil explosive, *x* =aqueous explosive.

The lysimeters were amended to lower levels of TNT and RDX than targeted (Table 1), because the comp-B used in the present case contained impurities of unusually high levels of TNB and HMX. This comp-B was composed on average of 31.7 % TNT, 41.8% RDX, 17.2% TNB, 9.3% HMX,

and a trace of MNX (Table 4). Documented HMX impurity of comp-B ranges from 1 to 10% and TNB has not been listed (Dontsova et al. 2006).

Table 4. Extractable explosives in the amended soil mixtures prior to the tests; contents per core and concentrations. Mean values and standard deviations are shown (N=6).

Comp-B Exposure	Extractable Explosives (g core^{-1})				
	TNT	TNB	RDX	MNX	HMX
Low	1.770 ± 0.527	0.910 ± 0.583	2.293 ± 0.340	0.002 ± 0	0.524 ± 0.227
Medium	3.633 ± 0.539	1.365 ± 0.373	4.944 ± 1.187	0.002 ± 0	1.101 ± 0.075
High	4.964 ± 0.397	3.754 ± 0.425	6.445 ± 0.909	0.003 ± 0	1.378 ± 0.132
(mg kg^{-1} soil dry wt)					
Low	23.359 ± 6.954	12.050 ± 7.720	30.259 ± 4.483	0.024 ± 0.007	6.907 ± 2.996
Medium	47.933 ± 7.116	18.075 ± 4.939	65.235± 15.660	0.032 ± 0.006	14.527 ± 0.987
High	65.493 ± 5.234	49.709 ± 5.628	85.030± 11.996	0.037 ± 0.012	18.176 ± 1.740

Plant responses to comp-B treatment

The *S. nutans* grass vegetation was well-established at the time when comp-B was amended and persisted throughout the 92-day exposure period (Figure 9). In contrast, the *A. retroflexus* forb vegetation did not take hold, despite reseeding, hormonal treatment and increasing seed-soil contact. Data pertaining to the forb-vegetated units were not further analyzed.

Temperature and soil moisture conditons were favorable for growth during the 92-day exposure period. Soil temperature fluctuated between 28 and 33 ^{o}C and did not vary with soil depth (Figure 10). Soil moisture patterns differed between vegetated and bare units. In the vegetated units, soil moisture was lowest in the top layer, fluctuating between 22 and 34%. It was higher and equal at 0.2 m and 0.43 m depth during the first 3-week period, and subsequently greatest in the 0.2-m depth layer - indicating moisture retainment at the bottom of the rhizosphere (Figure 10). In the bare units, soil moisture decreased from top to bottom 22% to 35% (Figure 10).

Shoot and plant (shoot plus root) biomass production of *S. nutans* after 92-day exposure were significantly affected by comp-B exposure ($p<0.001$; Table 5). The block effect for shoot and plant biomass was statistically significant, and, therefore, these data were analyzed using 'Block' as a covariate. Shoot and plant biomass increased with increasing comp-B

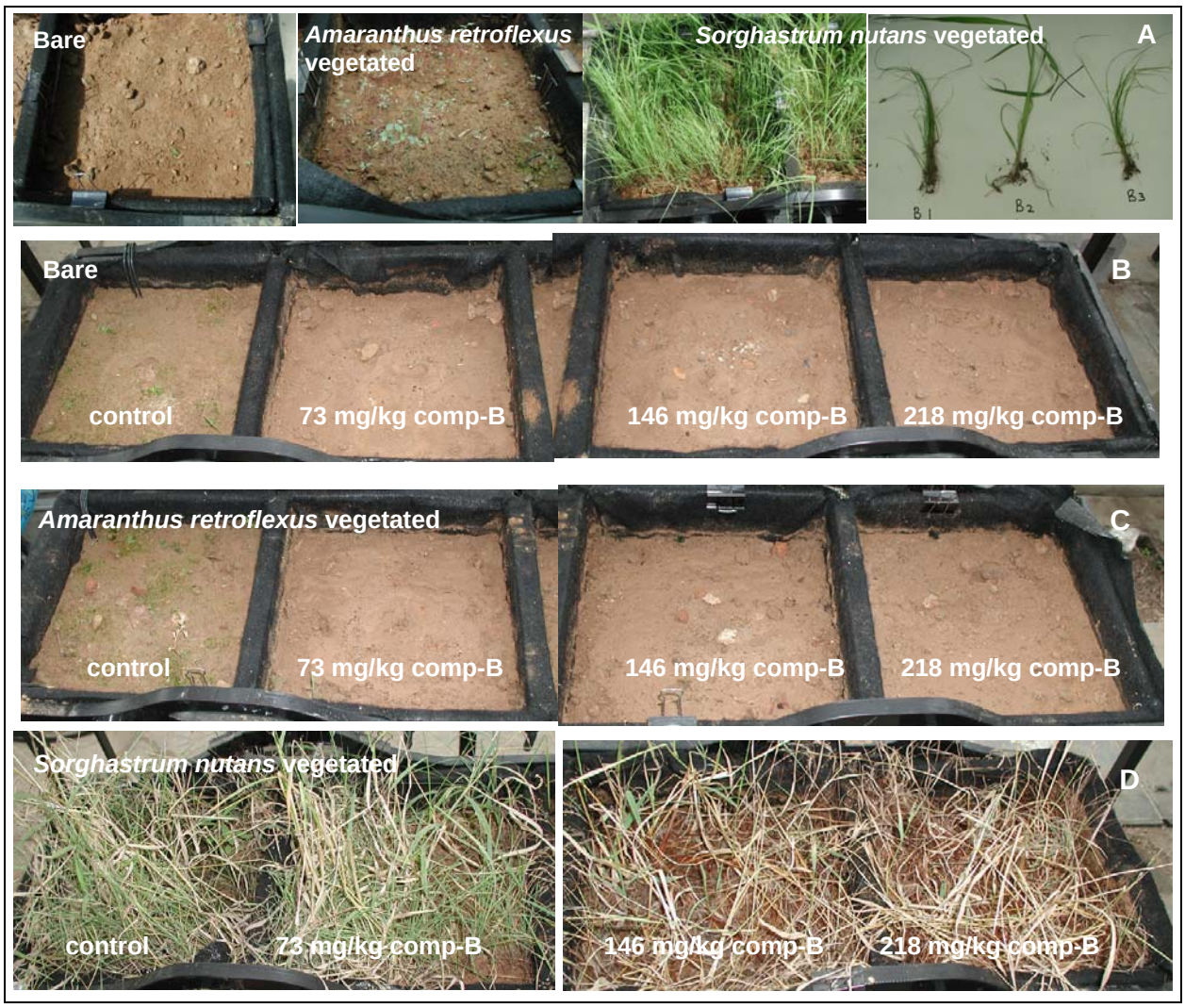

Figure 9. Bare and vegetated cores at the time of amendment (A) and after exposure to comp-B (block 3; B, C, D).

exposure up to a concentration of 73 mg kg^{-1} soil and decreased at higher exposures (Figure 11). Maximum shoot biomass was 235.3 g dry wt m^{-2} (Table 5). Root biomass was variable and fluctuated within a range of 41 to 56 g dry wt m^{-2}. Maximum plant biomass of 291.5 g dry wt m^{-2} was about half of the biomass produced under similar environmental, fertilized, conditions in the previous pot experiment (Best et al. 2008), and, therefore, nutrient limitation of growth was likely. Other plant biomass characteristics, including shoot:root ratio, root length and root diameter, were not affected by comp-B exposure (Table 5).

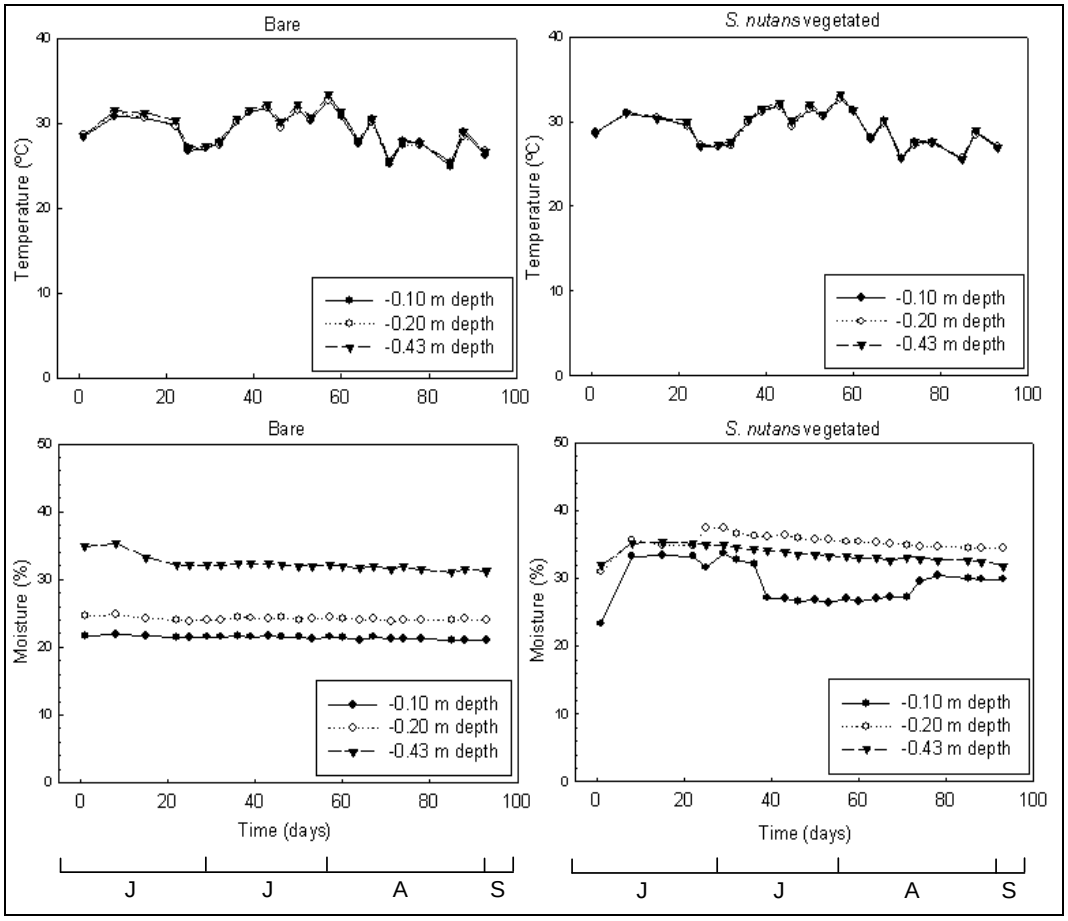

Figure 10. Temperature and moisture contents of control bare and *S. nutans* vegetated units.

Table 5. Biomass characteristics of *S. nutans* in response to 92 days of exposure to comp-B. Mean values and standard deviations are shown (N=3). Values that are followed by the same letter are not significantly different according to Fisher's Least Significant Difference procedure. ANOVA[1] results are listed.

Factor	Biomass Characteristics					
Comp-B Exposure	Shoot Biomass (g dry wt m^{-2})	Root Biomass (g dry wt m^{-2})	Plant Biomass (g dry wt m^{-2})	Shoot:Root Ratio	Root Length (m g^{-1} dry wt)	Root Diameter (mm)
Control	215.2 ± 126.2 abc	43.7 ± 7.6 ab	258.9 ± 127.9 ab	4.9 ± 3.0 a	3.2 ± 0 .4 a	0.65 ± 0.10 a
73 mg kg^{-1}	235.3 ± 69.8 c	56.3 ± 28.0 b	291.5 ± 93.1 c	4.6 ± 1.8 a	3.2 ± 2.4 a	0.66 ± 0.10 a
146 mg kg^{-1}	170.1 ± 44.8 b	46.9 ± 25.4 ab	255.9 ± 11.2 bc	4.2 ± 1.5 a	2.1 ± 0.2 a	0.70 ± 0.25 a
218 mg kg^{-1}	111.5 ± 32.6 a	40.7 ± 28.1 a	152.2 ± 36.1 a	5.1 ± 5.2 a	2.6 ± 0.7 a	0.59 ± 0.06 a
ANOVA[1]						
Factor		MS	F-ratio	p-value		
Comp-B exposure - shoot biomass		23012.0	7.43	<u><0.001</u>		
Comp-B exposure - root biomass		488.5	1.65	0.198		
Comp-B exposure - plant biomass		94406.9	9.19	<u><0.001</u>		
Comp-B exposure - shoot:root ratio		1.4	0.24	0.866		
Comp-B exposure - root length		2.4	0.66	0.582		
Comp-B exposure - root diameter		0.0	0.26	0.856		

[1] ANOVA results of plant biomass data, using 'Comp-B exposure' as factor and 'Block' as covariate. Underlining marks a statistically significant effect.

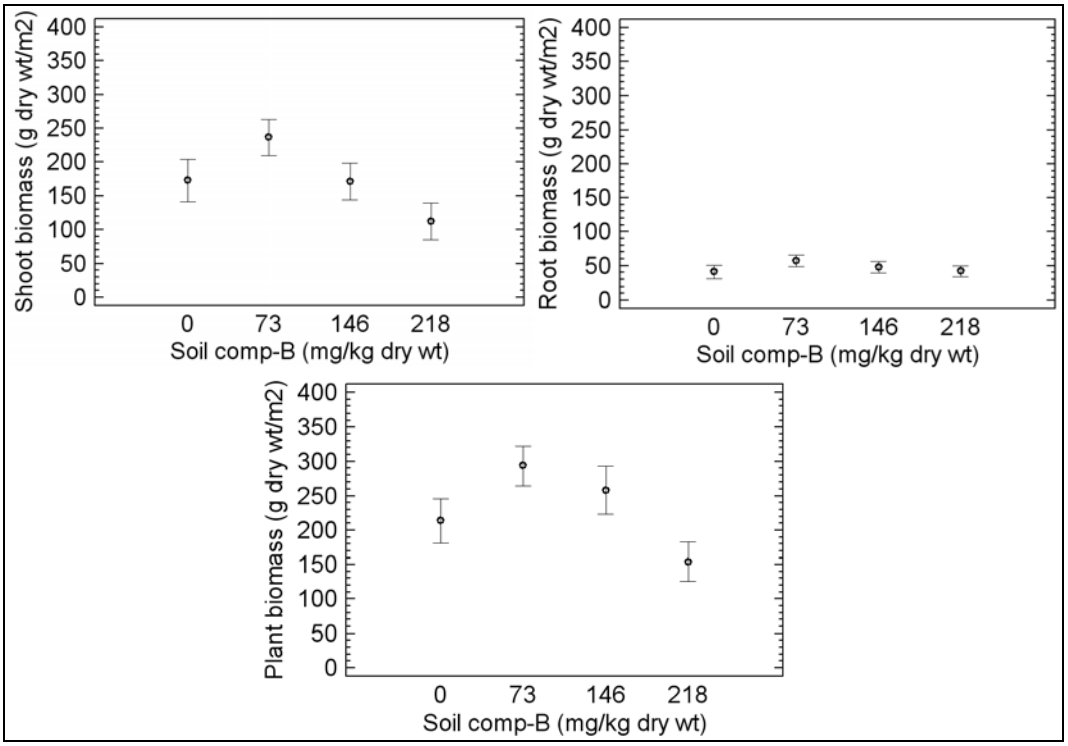

Figure 11. Shoot, root, and plant biomass of *S. nutans* in response to 92 days of exposure to comp-B. ANOVA results using 'Comp-B exposure' as factor and 'Block' as covariate. Mean values and standard errors of the mean.

The concentrations of TNT and the TNT degradation products 2ADNT and 4ADNT increased significantly with increasing comp-B exposure in the grass shoots and roots at the end of the 92-day period (Table 6, Figure 12). The levels of 2ADNT and 4ADNT usually exceeded those of TNT. The levels of RDX and MNX increased with comp-B exposure in shoots but not significantly, while the level of RDX significantly increased in roots (Table 6; Figure 13). MNX levels were usually on the order of 1% of the RDX levels.

Explosives were also determined in the monthly collected grass samples, and the data were statistically analyzed.

TNT, 2ADNT, and 4ADNT levels in shoots and roots of these samples were significantly affected by comp-B exposure (except for shoot 4ADNT) over the entire exposure period (Table 7). In addition, a time dependency of plant response in terms of accumulation of the explosives parent and degradation compounds was demonstrated. The 2ADNT and 4ADNT levels in shoots and roots were significantly affected by exposure period due to increasing TNT degradation over time, while the parent TNT level

Table 6. Extractable TNT, 2ADNT, 4ADNT, RDX and MNX concentrations in shoots and roots of *S. nutans* in response to 92 days of exposure to comp-B. Mean values and standard deviations are shown (N=3). Values that are followed by the same letter are not significantly different according to Fisher's Least Significant Difference procedure. ANOVA[1] results are listed.

Factor	Extractable Explosives Parent and Degradation Compounds in Plants				
Comp-B Exposure	TNT (mg kg^{-1} dry wt)	2ADNT (mg kg^{-1} dry wt)	4ADNT (mg kg^{-1} dry wt)	RDX (mg kg^{-1} dry wt)	MNX (mg kg^{-1} dry wt)
Shoots					
73 mg kg^{-1}	0.0 ± 0.0 a	78.5 ± 84.3 a	23.4 ± 40.5 a	3981.9 ± 832.9 a	47.1 ± 81.6 a
146 mg kg^{-1}	291.6 ± 318.0 b	502.5 ± 420.7 b	441.4 ± 384.2 b	4089.2 ± 283.1 a	47.2 ± 42.9 a
218 mg kg^{-1}	455.2 ± 329.8 c	774.1 ± 689.6 c	608.9 ± 541.0 b	4835.1 ± 2434.1 a	70.7 ± 61.7 a
Roots					
73 mg kg^{-1}	220.0 ± 381.1 a	165.5 ± 68.9 a	100.6 ±96.7 a	3461.6 ± 1495.7 a	8.5 ± 14.7 ab
146 mg kg^{-1}	667.4 ± 498.9 a	791.4 ± 504.8 b	840.7 ± 585.3 b	3314.2 ± 2640.7 a	10.0 ± 17.3 a
218 mg kg^{-1}	2566.9 ± 1709.6 b	1444.1 ± 1053.7 c	1420.3 ± 1114.4 c	5754.2 ± 3607.3 b	41.9 ± 72.6 b
ANOVA[1]					
Factor		MS	F-ratio	p-value	
Comp-B exposure - shoot-TNT		866170.0	10.84	<0.001	
Comp-B exposure - shoot-2ADNT		925834.0	11.43	<0.001	
Comp-B exposure - shoot-4ADNT		715804.0	12.52	<0.001	
Comp-B exposure - shoot-RDX		864735.0	0.81	0.459	
Comp-B exposure - shoot-MNX		1561.3	0.75	0.485	
Comp-B exposure - root-TNT		1.1 x 10^{7}	25.12	<0.001	
Comp-B exposure - root-2ADNT		0.3 x 10^{7}	24.80	<0.001	
Comp-B exposure - root-4ADNT		0.3 x 10^{7}	22.95	<0.001	
Comp-B exposure - root-RDX		1.5 x 10^{7}	7.37	0.003	
Comp-B exposure - root-MNX		4306.4	2.40	0.114	

[1] ANOVA results of plant biomass data, using 'Comp-B exposure' as factor and 'Block' as covariate. Underlining marks a statistically significant effect.

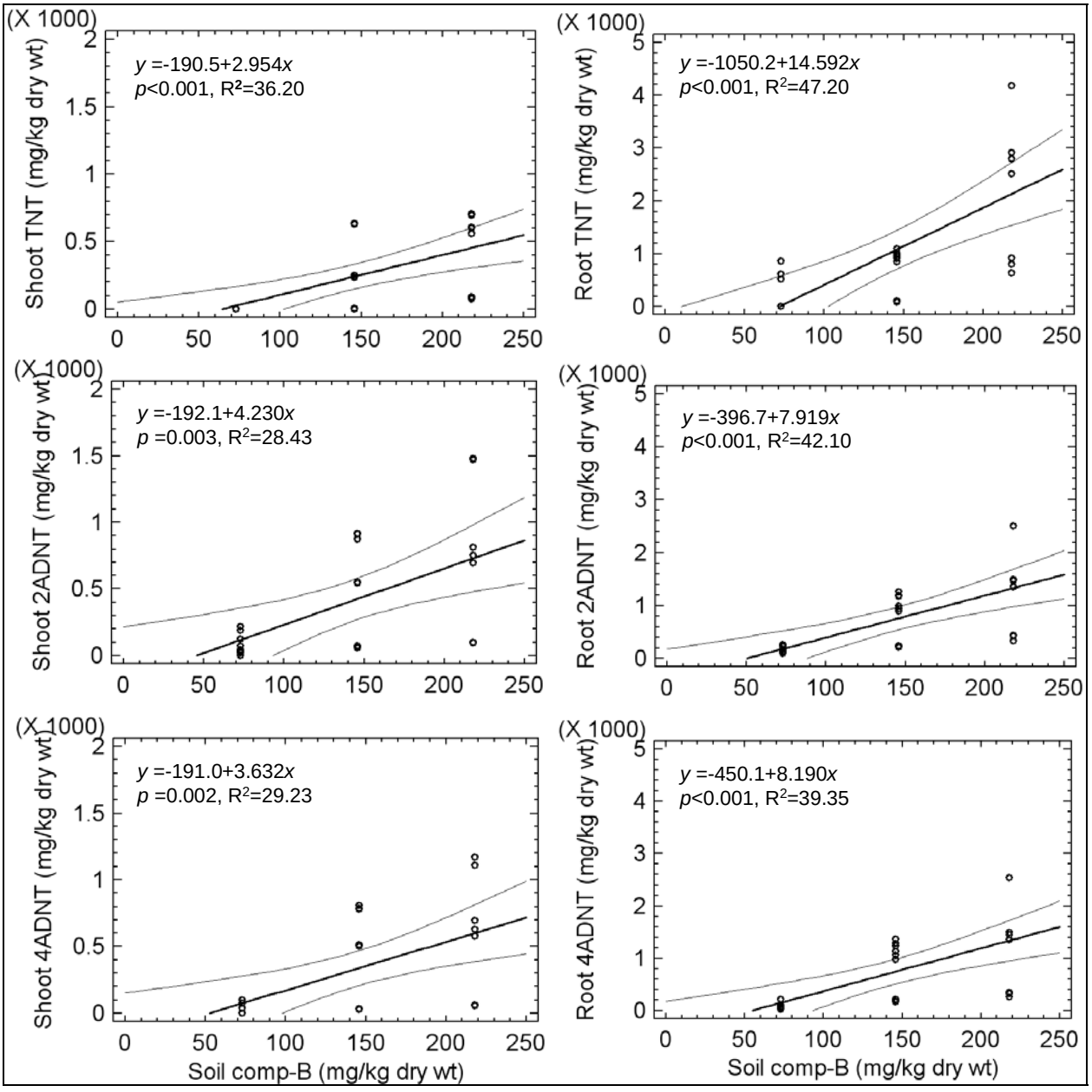

Figure 12. Extractable TNT, 2ADNT, and 4ADNT in shoots and roots of *S. nutans* in response to 92 days of exposure to comp-B. Regression lines and 95% confidence limits indicated; *y* = plant response, *x* = comp-B exposure.

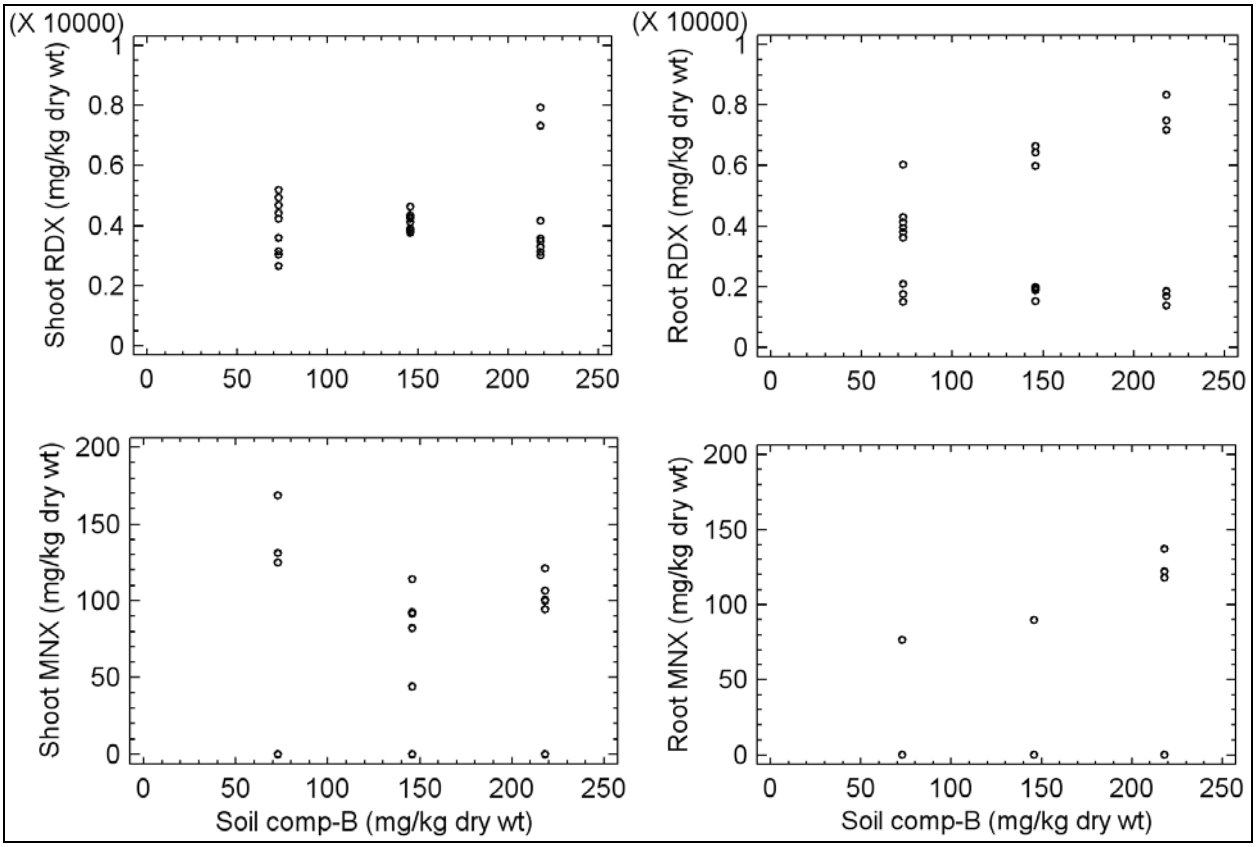

Figure 13. Extractable RDX and MNX in shoots and roots of *S. nutans* in response to 92 days of exposure to comp-B. No significant relationship between the shoot and root RDX and MNX concentrations and the comp-B exposures was identified using regression techniques (R^2 <10).

Table 7. ANOVA results of the effects of comp-B exposure[1], exposure period[1], and their interaction term on the extractable TNT, 2ADNT, 4ADNT, RDX and MNX concentrations in shoots and roots of *S. nutans* exposed for 28, 63, and 92 days to comp-B. Underlining marks a statistically significant effect. The effect of the interaction term was insignificant (data not shown). Measured values listed in Table A3.

Factor	MS	F-ratio	p-value
Comp-B Exposure (mg kg^{-1} soil-dry wt)			
Shoots			
Shoot-TNT	0.344 x 10^6	11.03	<0.001
Shoot-2ADNT	0.278 x 10^6	4.33	0.021
Shoot-4ADNT	0.152 x 10^6	3.18	0.054
Shoot-RDX	0.221 x 10^6	0.25	0.776
Shoot-MNX	1525.81	0.80	0.456
Roots			
Root-TNT	6.272x 10^6	15.19	<0.001
Root-2ADNT	1.472x 10^6	11.99	<0.001
Root-4ADNT	0.800 x 10^6	4.98	0.012
Root-RDX	6.124x 10^6	2.41	0.105
Root-MNX	2705.36	1.85	0.172

Factor	MS	F-ratio	p-value
Exposure period (d)			
	Shoots		
Shoot-TNT	45370.3	1.45	0.249
Shoot-2ADNT	0.570 x 10^6	8.86	<0.001
Shoot-4ADNT	0.416 x 10^6	8.73	<0.001
Shoot-RDX	34.694x 10^6	39.72	<0.001
Shoot-MNX	10452.6	5.51	0.008
	Roots		
Root-TNT	0.711 x 10^6	1.72	0.193
Root-2ADNT	0.729 x 10^6	5.95	0.006
Root-4ADNT	1.371x 10^6	8.54	0.001
Root-RDX	3.364x 10^6	11.95	<0.001
Root-MNX	1670.78	1.14	0.330

[1] Block used as covariate.

was not (Table 7). RDX and MNX levels in shoots and roots were not significantly affected by comp-B exposure over the entire exposure period. However, shoot RDX and MNX and root RDX were significantly affected by exposure period, indicating that accumulation of RDX increased with time and that degradation of RDX resulting in increased MNX levels largely occurred in the shoots (Table 7). The effect of the interaction term 'Comp-B exposure' x 'Exposure period' was not significant.

Water balances and explosives loss with leachates

Water balances were prepared for all lysimeter units (Table 2). These balances show that rainfall water exceeded the initial amount of water contained in the moist soil cores by a factor of 7 to 8 and water removed with the leachates comprised only 20 to 34% of rainfall. Evapotranspiration ranged from 1.9 to 2.4 L m^{-2} d^{-1}, which is in the same range as determined in the previous pot experiment (Best et al. 2008).

The concentrations of both TNT and RDX in the leachates were significantly affected by comp-B exposure and by vegetative cover, and that of RDX also by exposure period (Table 8). The block effect was statistically significant, and, therefore, these data were analyzed using 'Block' as a covariate.

Table 8. ANOVA of the effects of 'Comp-B exposure[1]', 'Exposure period[1]', and 'Vegetative cover[1]' on the TNT and RDX concentrations in the leachates. Underlining marks a statistically significant effect.

Factor	MS	F-ratio	p-value
TNT (mg L^{-1})			
Comp-B exposure (mg kg^{-1} dry wt)	45.28	21.60	<0.001
Exposure period (d)	2.55	1.22	0.221
Vegetative cover (nominal)	31.96	15.25	<0.001
RDX (mg L^{-1})			
Comp-B exposure (mg kg^{-1} dry wt)	57.05	12.96	<0.001
Exposure period (d)	9.04	2.05	0.002
Vegetative cover (nominal)[1]	1024.66	23.79	<0.001

[1] Block used as covariate.

The concentrations of TNT and RDX compounds and all degradation compounds, except 24DANT, were usually greater and more variable in the leachates from the vegetated units than in those of the bare units (Figures 14 and 15). The total explosives parent and degradation compound contents of the leachates of the vegetated units were also greater than those of the bare units (Table 9). Leachate TNT contents were 3.33, 3.99, and 10.06 mg from the vegetated units and 1.11, 1.18, and 4.07 mg from the bare units exposed to 73, 146, and 218 mg comp-B, respectively. RDX contents were 27.36, 15.78, and 16.93 mg from the vegetated units, and 5.77, 5.62, and 10.92 mg from the bare units. The following patterns were identified in the leachates. In leachates from the vegetated units, TNT concentrations started at a relatively low level, peaked after about one week, and gradually decreased subsequently (Figure 16). 2ADNT and 4ADNT peaks followed TNT peaks with a two-week delay, and 24DANT followed ADNT peaks with a four-week delay. RDX showed a different pattern. RDX started at a relatively low level, increased to a maximum after four weeks, and decreased subsequently very slowly (Figure 17). MNX started at a level below detection, started to increase sigmoidally after three weeks, while DNX exhibited the same pattern as MNX and followed MNX with a four-week delay. Similar patterns were found in leachates from the bare units. However, the latter patterns suffered from highly variable levels determined in leachates collected after 15-day exposures, because of leakage of one of the lysimeter units.

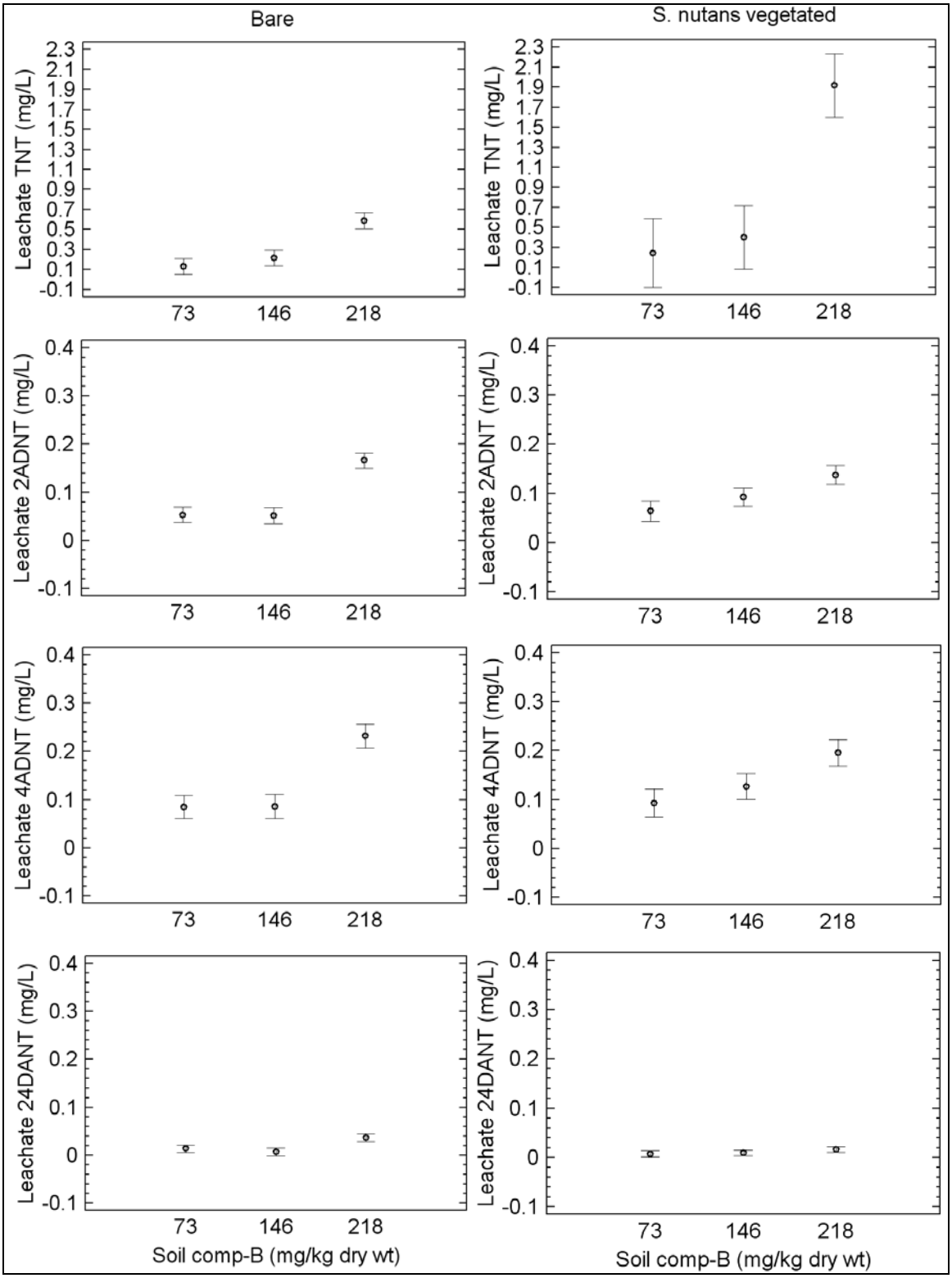

Figure 14. Concentration patterns over exposure of TNT and its degradation compounds in leachates collected from bare and *S. nutans* vegetated units exposed to comp-B. Mean values and standard errors of the mean generated by ANOVA, using 'Comp-B exposure' as factor, and 'Exposure period' and 'Block' as covariates.

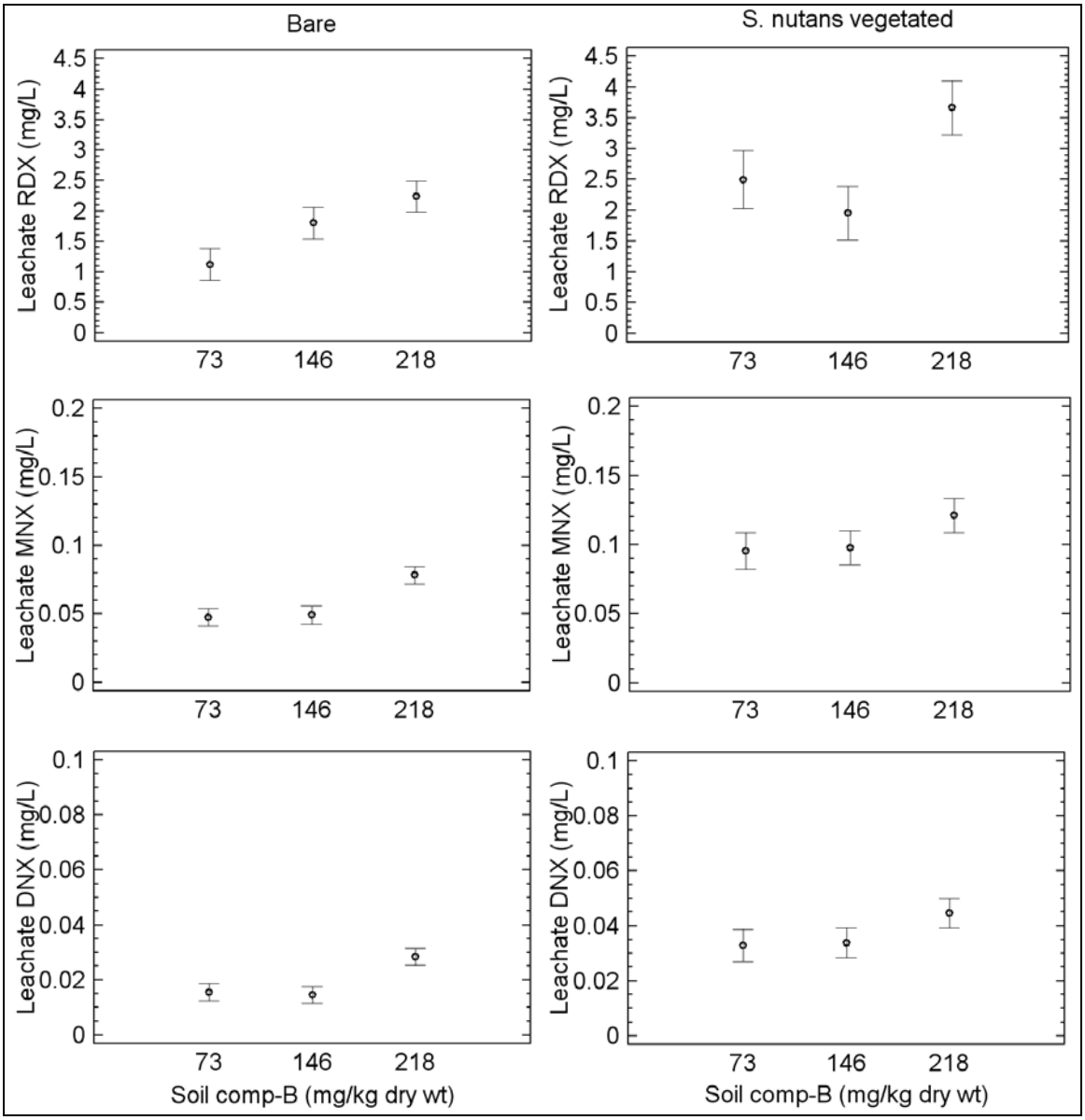

Figure 15. Concentration patterns over exposure of RDX and its degradation compounds in leachates collected from bare and *S. nutans* vegetated units exposed to comp-B. Mean values and standard errors of the mean generated by ANOVA, using 'Comp-B exposure' as factor, and 'Exposure period' and 'Block' as covariates.

Table 9. TNT, 2ADNT, 4ADNT, RDX and MNX contents of leachates collected from bare and vegetated soil cores during 92 days of exposure to comp-B. Measured values, means and standard deviations are shown (N=3).

Factor	TNT, RDX Parent and Degradation Compounds in Leachates					
Comp-B Exposure	Block	TNT (mg)	2ADNT (mg)	4ADNT (mg)	RDX (mg)	MNX (mg)
Bare						
73 mg kg^{-1}	1	0.05	0.04	0.05	0.86	0.04
73 mg kg^{-1}	2	2.74	0.43	0.65	8.11	0.36
73 mg kg^{-1}	3	0.56	0.14	0.24	8.36	0.32
Mean ± SD		1.11 ± 1.43	0.20 ± 0.20	0.32 ± 0.31	5.77 ± 4.26	0.24 ± 0.18
146 mg kg^{-1}	1	1.19	0.08	0.10	2.96	0.22
146 mg kg^{-1}	2	0.51	0.26	0.40	5.18	0.40
146 mg kg^{-1}	3	1.84	0.17	0.32	8.71	0.14
Mean ± SD		1.18 ± 0.67	0.17 ± 0.09	0.27 ± 0.16	5.62 ± 2.90	0.25 ± 0.13
218 mg kg^{-1}	1	0.48	0.27	0.37	5.64	0.56
218 mg kg^{-1}	2	5.78	1.57	2.04	18.00	0.69
218 mg kg^{-1}	3	5.94	0.41	0.50	9.16	0.28
Mean ± SD		4.07 ± 3.11	0.75 ± 0.71	0.97 ± 0.93	10.92 ± 6.36	0.51 ± 0.21
S. nutans vegetated						
73 mg kg^{-1}	1	0.47	0.25	0.36	7.52	0.61
73 mg kg^{-1}	2	0.22	0.09	0.17	3.28	0.03
73 mg kg^{-1}	3	9.31	1.36	2.02	71.28	1.92
Mean ± SD		3.33 ± 5.17	0.57 ± 0.69	0.85 ± 1.02	27.36 ± 38.10	0.85 ± 0.97
146 mg kg^{-1}	1	4.31	1.82	2.30	22.44	1.53
146 mg kg^{-1}	2	5.14	0.91	1.05	12.59	0.41
146 mg kg^{-1}	3	2.53	0.44	0.63	12.33	0.69
Mean ± SD		3.99 ± 1.33	1.06 ± 0.70	1.33 ± 0.87	15.78 ± 5.76	0.88 ± 0.59
218 mg kg^{-1}	1	0.29	0.27	0.37	6.99	0.87
218 mg kg^{-1}	2	16.99	1.85	1.79	24.56	1.22
218 mg kg^{-1}	3	12.91	0.71	1.03	19.24	0.34
Mean ± SD		10.06 ± 8.71	0.95 ± 0.81	1.06 ± 0.71	16.93 ± 9.01	0.81 ± 0.44

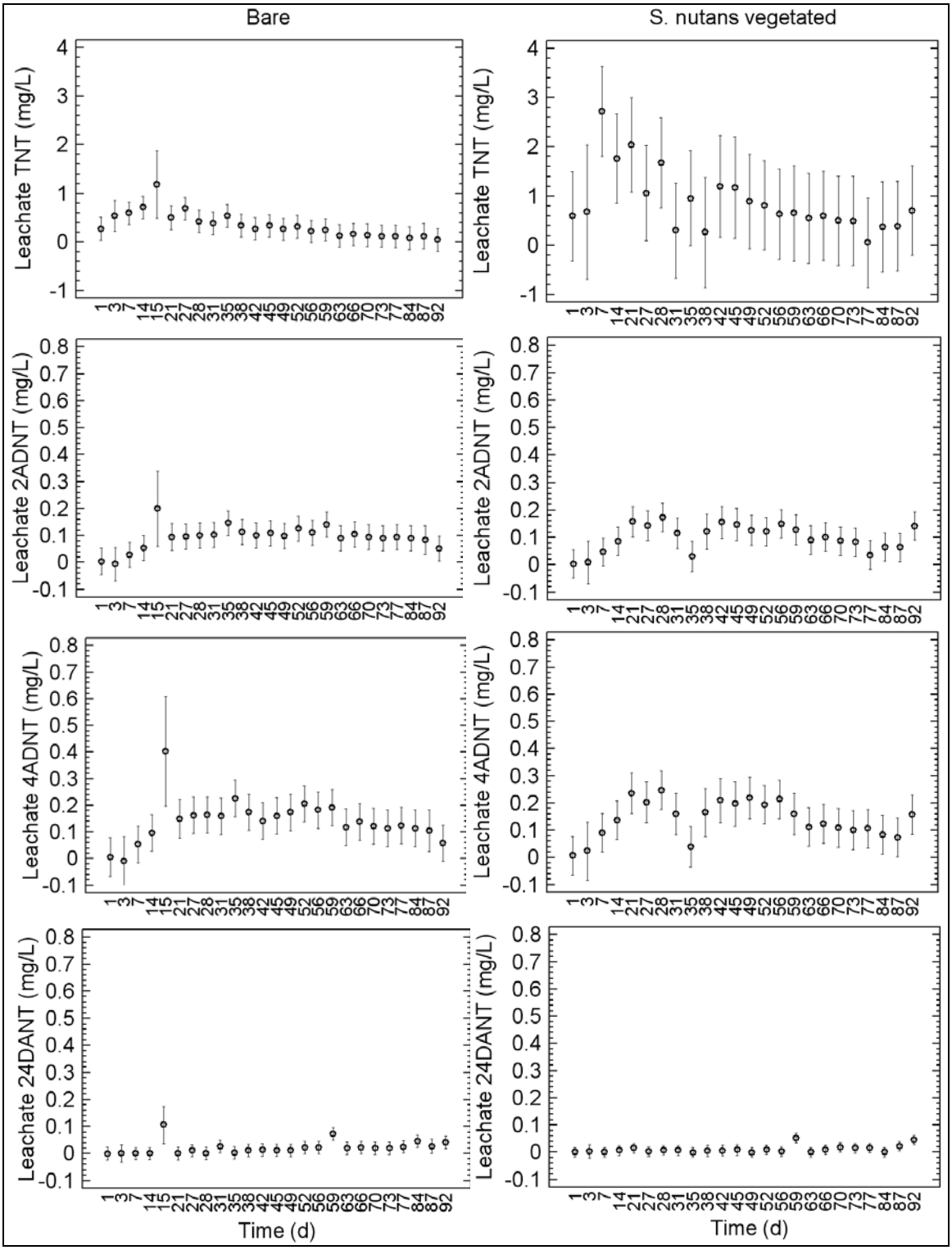

Figure 16. Concentration patterns over time of TNT and its degradation compounds in leachates collected from bare and *S. nutans* vegetated units exposed to comp-B. Mean values and standard errors of the mean generated by ANOVA, using 'Exposure period' (=time) as factor, and 'Comp-B exposure' and 'Block' as covariates.

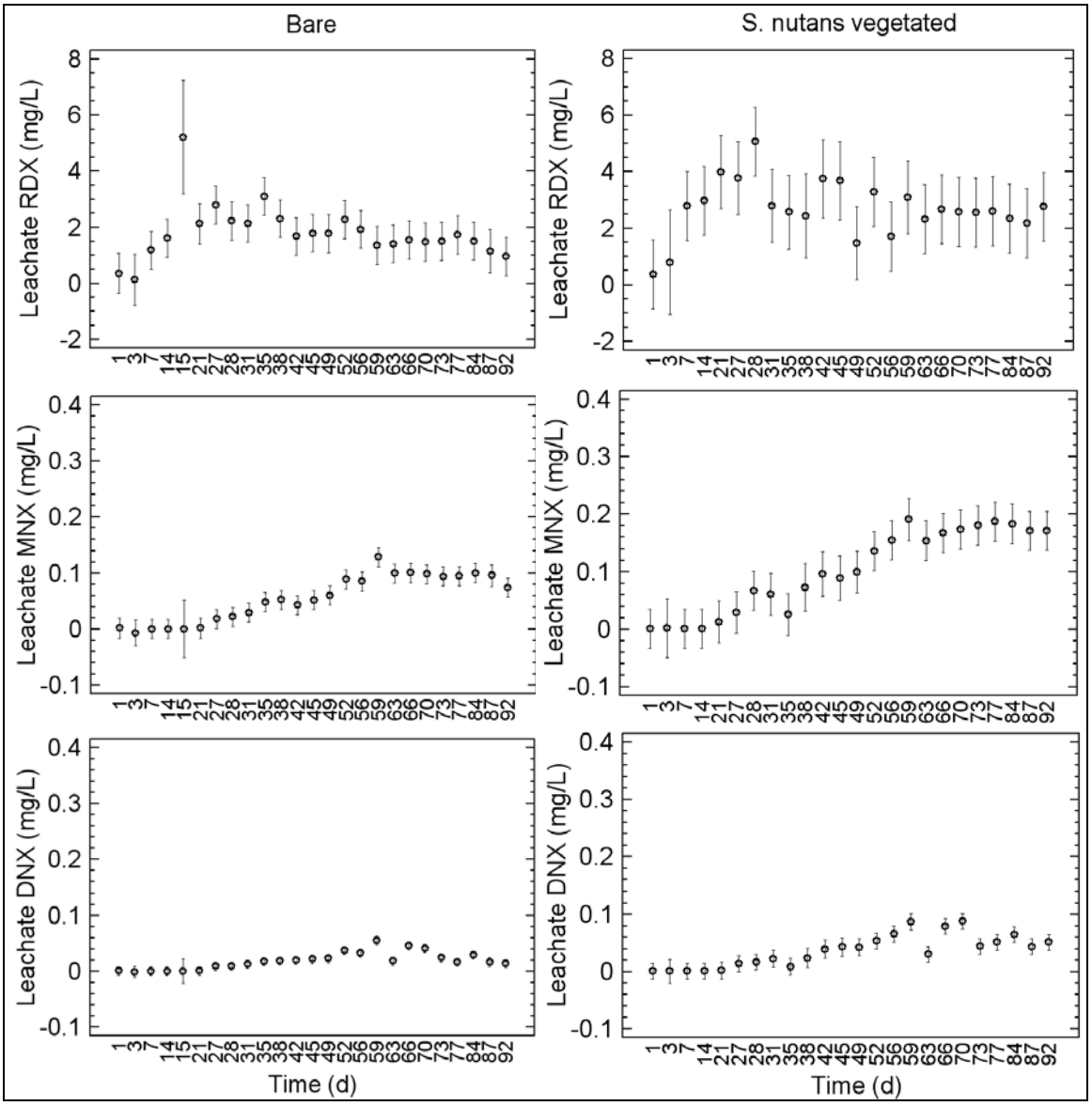

Figure 17. Concentration patterns over time of RDX and its degradation compounds in leachates collected from bare and *S. nutans* vegetated units exposed to comp-B. Mean values and standard errors of the mean generated by ANOVA, using 'Exposure period' (=time) as factor, and 'Comp-B exposure' and 'Block' as covariates.

Explosives distribution in vegetated and bare soil cores exposed to comp-B treatment

The concentrations of TNT and RDX in the soil after 92 days of exposure to comp-B were significantly affected by comp-B exposure and by layer, but not by vegetative cover (Table 10). The block effect was statistically significant, and, therefore, these data were analyzed using 'Block' as a covariate.

Table 10. ANOVA of the effects of 'Comp-B exposure'[1], 'Layer'[1], and 'Vegetative cover'[1] on the extractable TNT and RDX concentrations in soil cores after 92 days of exposure to comp-B. Underlining marks a statistically significant effect.

Factor	MS	F-ratio	p-value
TNT (mg kg^{-1} soil dry wt)			
Comp-B exposure (mg kg-1 dry wt)	6342.58	15.22	<0.001
Layer (nominal)	6882.34	16.51	<0.001
Vegetative cover (nominal)	157.73	0.38	0.541
RDX (mg kg^{-1} soil dry wt)			
Comp-B exposure (mg kg^{-1} dry wt)	24304.4	14.61	<0.001
Layer (nominal)	41932.8	25.20	<0.001
Vegetative cover (nominal)	1421.69	0.85	0.360

[1] Block used as covariate.

Because vegetative cover did not significantly affect the soil explosives concentration, the data pertaining to the vegetated and bare units were pooled to obtain regressions of the extracted soil concentrations over comp-B exposure for the three soil layers: top, mid, and bottom layer. Extracted soil concentrations of TNT and RDX were linearly related to comp-B exposure, with levels decreasing in the order top>mid>bottom layer (Figure 18). The levels of TNT were about 60% less than those of RDX, and reflected the same ratio between TNT and RDX as found in the amendments. As in the leachates, the variability in the explosives concentrations in soil of the vegetated units was greater than in soil of the bare units (Figure 18, Tables 11 and 12). Regarding degradation compounds, 2ADNT and 4ADNT were present at lower levels than their parent TNT, and MNX at a trace level (Table 12). 24DANT and DNX, identified in the leachates, were not found in the soil. Since 24DANT and DNX were also not identified in the plant materials, this may indicate that the transformation step from which these products resulted took place in the soil-water component of the systems. Large portions of the amended explosives parent compounds were recovered from the 92-day exposed soil cores, with extractable TNT accounting for maximally 35% and 39% and for extractable RDX accounting for 79% and 66% in the vegetated and bare units, respectively (Tables 13-17).

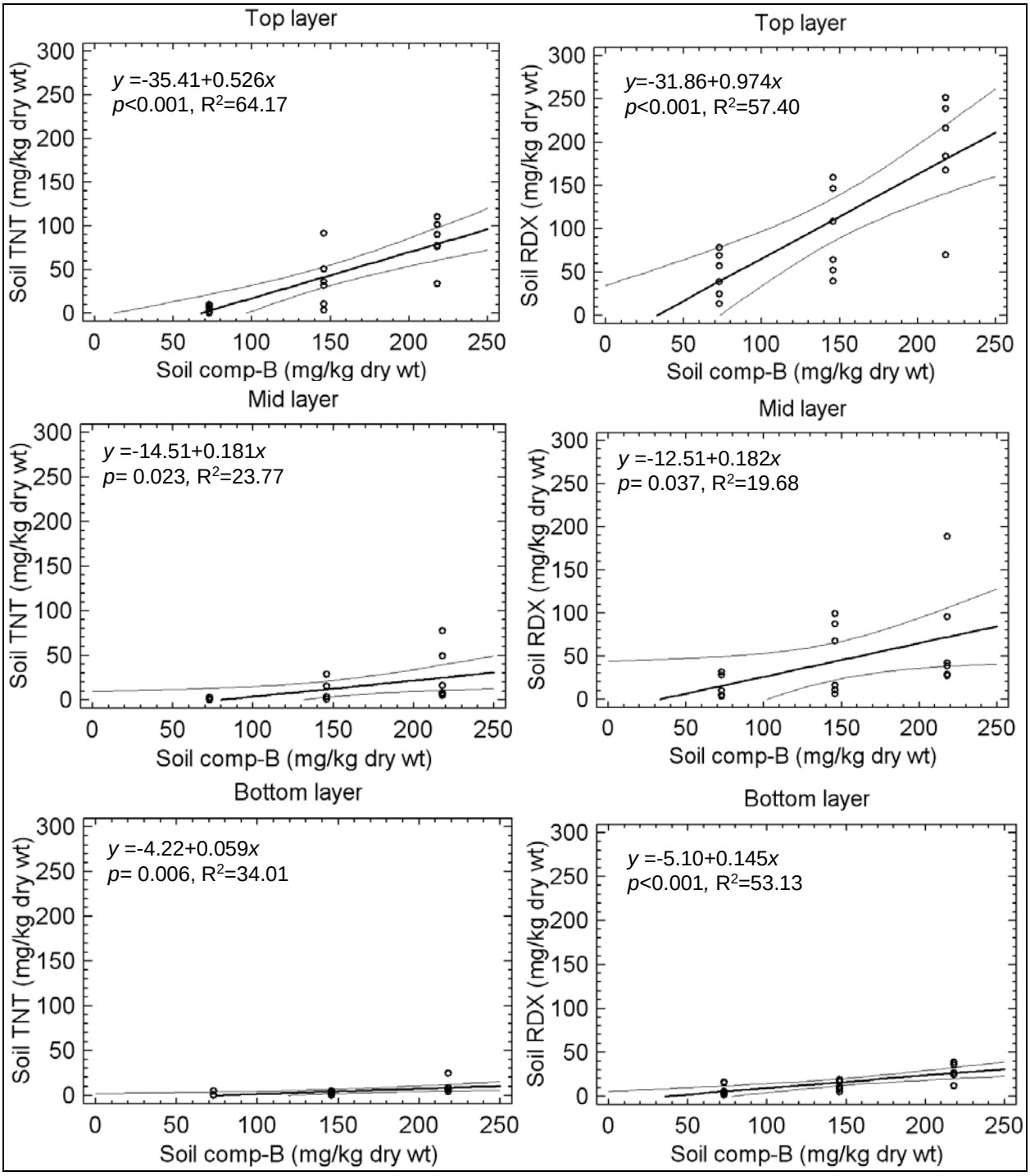

Figure 18. Extractable TNT and RDX in top, mid, and bottom soil layers after 92 days of exposure to comp-B. Regression lines and 95% confidence limits indicated; *y* = concentration exposed soil layer, *x* = comp-B exposure.

Table 11. Extractable TNT, 2ADNT, and 4ADNT concentrations in soil cores after 92 days of exposure to comp-B. Mean values and standard deviations are shown (N=3).

Factor	Extractable TNT parent and degradation compounds in soil				
Comp-B exposure	Initial TNT (mg kg^{-1} dry wt)	Layer	Final TNT (mg kg^{-1} dry wt)	Final 2ADNT (mg kg^{-1} dry wt)	Final 4ADNT (mg kg^{-1} dry wt)
Bare					
73 mg kg^{-1}		Top	6.2 ± 2.0	0.9 ± 0.7	0.3 ± 0.5
73 mg kg^{-1}		Mid	1.2 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
73 mg kg^{-1}		Bottom	1.9 ± 2.7	0.0 ± 0.0	0.0 ± 0.0
73 mg kg^{-1}	23.4 ± 7.0	Total			
146 mg kg^{-1}		Top	52.9 ± 33.6	1.8 ± 0.8	0.8 ± 0.6
146 mg kg^{-1}		Mid	5.5 ± 8.6	0.2 ± 0.3	0.0 ± 0.0
146 mg kg^{-1}		Bottom	1.3 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
146 mg kg^{-1}	47.9 ± 7.1	Total			
218 mg kg^{-1}		Top	73.5 ± 38.4	1.3 ± 0.3	0.4 ± 0.2
218 mg kg^{-1}		Mid	32.9 ± 39.2	0.6 ± 0.3	0.0 ± 0.0
218 mg kg^{-1}		Bottom	12.3 ± 10.8	0.2 ± 0.2	0.0 ± 0.0
218 mg kg^{-1}	65.5 ± 5.2	Total			
S. nutans vegetated					
73 mg kg^{-1}		Top	4.0 ± 5.1	2.1 ± 1.0	1.1 ± 1.0
73 mg kg^{-1}		Mid	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
73 mg kg^{-1}		Bottom	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
73 mg kg^{-1}	23.4± 7.0	Total			
146 mg kg^{-1}		Top	21.6 ± 25.6	1.1 ± 0.6	0.5 ± 0.3
146 mg kg^{-1}		Mid	10.9 ± 15.5	0.5 ± 0.4	0.0 ± 0.0
146 mg kg^{-1}		Bottom	2.6 ± 2.4	0.0 ± 0.0	0.0 ± 0.0
146 mg kg^{-1}	47.9 ± 7.1	Total			
218 mg kg^{-1}		Top	89.4 ± 12.0	2.0 ± 1.2	1.0 ± 0.8
218 mg kg^{-1}		Mid	21.1 ± 24.4	0.7 ± 0.4	0.1 ± 0.1
218 mg kg^{-1}		Bottom	6.9 ± 1.3	0.5 ± 0.4	0.0 ± 0.0
218 mg kg^{-1}	65.5 ± 5.2	Total			

Table 12. Extractable RDX and MNX concentrations in soil cores after 92 days of exposure to comp-B. Mean values and standard deviations are shown (N=3).

Factor	Extractable RDX Parent and Degradation Compounds in Soil			
Comp-B Exposure	Initial RDX (mg kg^{-1} dry wt)	Layer	Final RDX (mg kg^{-1} dry wt)	Final MNX (mg kg^{-1} dry wt)
Bare				
73 mg kg^{-1}		Top	25.3 ± 12.4	0.0 ± 0.0
73 mg kg^{-1}		Mid	23.2 ± 11.6	0.0 ± 0.0
73 mg kg^{-1}		Bottom	12.5 ± 5.9	0.0 ± 0.0
73 mg kg^{-1}	30.3 ± 4.5	Total		
146 mg kg^{-1}		Top	98.4 ± 54.3	0.0 ± 0.0
146 mg kg^{-1}		Mid	29.9 ± 32.9	0.0 ± 0.0
146 mg kg^{-1}		Bottom	10.3 ± 7.3	0.0 ± 0.0
146 mg kg^{-1}	65.2 ± 15.7	Total		
218 mg kg^{-1}		Top	151.3 ± 74.7	0.2 ± 0.4
218 mg kg^{-1}		Mid	86.8 ± 88.8	0.3 ± 0.5
218 mg kg^{-1}		Bottom	25.6 ± 13.4	0.0 ± 0.0
218 mg kg^{-1}	85.0 ± 12.0	Total		
S. nutans vegetated				
73 mg kg^{-1}		Top	68.2 ± 10.8	0.5 ± 0.8
73 mg kg^{-1}		Mid	4.3 ± 0.9	0.0 ± 0.1
73 mg kg^{-1}		Bottom	3.0 ± 1.4	0.1 ± 0.1
73 mg kg^{-1}	30.3 ± 4.5	Total		
146 mg kg^{-1}		Top	91.9 ± 58.5	0.0 ± 0.1
146 mg kg^{-1}		Mid	65.5 ± 48.1	0.0 ± 0.0
146 mg kg^{-1}		Bottom	12.5 ± 3.6	0.0 ± 0.0
146 mg kg^{-1}	65.2 ± 15.7	Total		
218 mg kg^{-1}		Top	224.7 ± 36.2	0.4 ± 0.7
218 mg kg^{-1}		Mid	53.8 ± 36.6	0.0 ± 0.0
218 mg kg^{-1}		Bottom	31.8 ± 6.6	0.0 ± 0.0
218 mg kg^{-1}	85.0 ± 12.0	Total		

Table 13. Extractable TNT, 2ADNT, 4ADNT contents in soil cores after 92 days of exposure to comp-B. Mean values and standard deviations are shown (N=3).

Factor	Extractable TNT Parent and Degradation Compounds in Soil				
Comp-B Exposure	Initial TNT (mg core^{-1})	Layer	Final TNT (mg core^{-1})	Final 2ADNT (mg core^{-1})	Final 4ADNT (mg core^{-1})
Bare					
73 mg kg^{-1}		Top	77.7 ± 25.2	11.0 ± 9.0	3.3 ± 5.6
73 mg kg^{-1}		Mid	14.9 ± 13.0	0.0 ± 0.0	0.0 ± 0.0
73 mg kg^{-1}		Bottom	95.3 ± 139.5	0.0 ± 0.0	0.0 ± 0.0
73 mg kg^{-1}	1770 ± 527	Total	187.9 ± 162.5	11.0 ± 9.0	3.3 ± 5.6
146 mg kg^{-1}		Top	658.1 ± 418.7	22.0 ± 9.6	9.6 ± 7.9
146 mg kg^{-1}		Mid	68.0 ± 106.8	2.5 ± 4.4	0.0 ± 0.0
146 mg kg^{-1}		Bottom	66.4 ± 57.5	0.0 ± 0.0	0.0 ± 0.0
146 mg kg^{-1}	3633 ± 539	Total	792.5 ± 565.0	24.5 ± 13.9	9.6 ± 7.9
218 mg kg^{-1}		Top	914.6 ± 478.0	15.6 ± 3.3	4.6 ± 2.7
218 mg kg^{-1}		Mid	409.0 ± 487.8	8.0 ± 4.1	0.0 ± 0.0
218 mg kg^{-1}		Bottom	623.6 ± 551.3	11.0 ± 12.1	1.2 ± 2.0
218 mg kg^{-1}	4964± 397	Total	1947.2 ± 342.1	34.6 ± 6.4	5.8 ± 4.5
S. nutans vegetated					
73 mg kg^{-1}		Top	49.3 ± 63.5	25.6 ± 12.6	13.1 ± 12.8
73 mg kg^{-1}		Mid	0.8 ± 0.9	0.0 ± 0.0	0.0 ± 0.0
73 mg kg^{-1}		Bottom	7.5 ± 12.1	0.0 ± 0.0	0.0 ± 0.0
73 mg kg^{-1}	1770 ± 527	Total	57.6 ± 61.7	25.6 ± 12.6	13.1 ± 12.8
146 mg kg^{-1}		Top	268.7 ± 319.2	13.4 ± 8.0	6.8 ± 3.2
146 mg kg^{-1}		Mid	135.3 ± 192.8	5.7 ± 5.0	0.0 ± 0.0
146 mg kg^{-1}		Bottom	134.0 ± 120.2	1.0 ± 1.8	0.0 ± 0.0
146 mg kg^{-1}	3633 ± 539	Total	538.0 ± 540.5	20.1 ± 5.2	6.8 ± 3.2
218 mg kg^{-1}		Top	113.5 ± 149.2	24.3 ± 15.1	13.0 ± 10.2
218 mg kg^{-1}		Mid	262.7 ± 304.3	8.6 ± 5.0	1.0 ± 0.9
218 mg kg^{-1}		Bottom	353.5 ± 66.4	23.1 ± 20.9	0.0 ± 0.0
218 mg kg^{-1}	4964± 397	Total	1729.7 ± 304.1	56.0 ± 31.2	14.0 ± 10.7

Table 14. Extractable RDX, MNX, and DNX contents in soil cores after 92 days of exposure to comp-B. Mean values and standard deviations are shown (N=3).

Factor	Extractable RDX Parent and Degradation Compounds in Soil			
Comp-B Exposure	Initial RDX (mg core^{-1})	Layer	Final RDX (mg core^{-1})	Final MNX (mg core^{-1})
Bare				
73 mg kg^{-1}		Top	315.1 ± 154.8	0.0 ± 0.0
73 mg kg^{-1}		Mid	289.0 ± 144.3	0.0 ± 0.0
73 mg kg^{-1}		Bottom	635.9 ± 301.7	0.0 ± 0.0
73 mg kg^{-1}	2293 + 340	Total	1240.0 ± 341.0	0.0 ± 0.0
146 mg kg^{-1}		Top	1224.9 ± 676.4	0.0 ± 0.0
146 mg kg^{-1}		Mid	372.7 ± 410.1	0.0 ± 0.0
146 mg kg^{-1}		Bottom	523.9 ± 370.5	0.0 ± 0.0
146 mg kg^{-1}	4944 ± 1187	Total	2121.5 ± 14108.4	0.0 ± 0.0
218 mg kg^{-1}		Top	1884.0 ± 929.4	3.0 ± 5.2
218 mg kg^{-1}		Mid	1080.5 ± 1106.1	3.6 ± 6.3
218 mg kg^{-1}		Bottom	1305.0 ± 681.5	0.0 ± 0.0
218 mg kg^{-1}	6445 ± 909	Total	4269.4 ± 1335.0	6.6 ± 5.8
S. nutans vegetated				
73 mg kg^{-1}		Top	848.7 ± 134.4	5.6 ± 9.7
73 mg kg^{-1}		Mid	53.5 ± 10.7	0.5 ± 0.9
73 mg kg^{-1}		Bottom	152.0 ± 69.5	2.6 ± 4.6
73 mg kg^{-1}	2293 + 340	Total	1054.1 ± 87.9	8.8 ± 15.2
146 mg kg^{-1}		Top	1143.8 ± 728.3	0.4 ± 0.8
146 mg kg^{-1}		Mid	816.1 ± 598.6	0
146 mg kg^{-1}		Bottom	637.4 ± 181.7	0
146 mg kg^{-1}	4944 + 1187		2597.3 ± 1098.4	0.4 ± 0.8
218 mg kg^{-1}		Top	2797.7 ± 450.6	4.7 ± 8.2
218 mg kg^{-1}		Mid	669.7 ± 455.4	0.0 ± 0.0
218 mg kg^{-1}		Bottom	1620.8 ± 333.5	0.0 ± 0.0
218 mg kg^{-1}	6445 ± 909	Total	5088.2 ± 565.2	4.7 ± 8.2

Table 15. TNT mass balances of soil-plant systems, in mg per core and % of initial extractable comp-B parent compound (TNT) per core. Measured values, means and standard deviations are shown (N=3). NA=not applicable.

Comp-B Exposure	Block	In. Soil-TNT (mg core^{-1})	Final Soil-TNT		Final Plant-TNT		Leach-TNT		TNT-loss Other Processes	
			(mg core^{-1})	(%)	(mg core^{-1})	(%)	(mg core^{-1})	(%)	(mg core^{-1})	(%)
Bare										
73 mg kg^{-1}	1	1770	128.9	7.3	NA	NA	0.0	0.0	1641.1	92.7
73 mg kg^{-1}	2	1770	371.6	21.0	NA	NA	2.7	0.2	1395.7	78.9
73 mg kg^{-1}	3	1770	63.1	3.6	NA	NA	0.6	0.0	1706.3	96.4
Mean ± SD		1770 ± 527	187.9 ± 162.5	10.6 ± 9.2			1.1 ± 0.1	0.1±0.1	1581.0±163.8	89.3 ± 9.3
146 mg kg^{-1}	1	3633	480.8	13.2	NA	NA	1.2	0.0	3150.0	86.7
146 mg kg^{-1}	2	3633	452.0	12.4	NA	NA	0.5	0.0	3180.5	87.5
146 mg kg^{-1}	3	3633	1444.6	39.8	NA	NA	1.8	0.1	2186.6	60.2
Mean ± SD		3633 ± 539	792.5 ± 564.9	21.8 ±15.6			1.2 ± 0.7	0.0±0.0	2839.4±565.5	78.2 ± 15.6
218 mg kg^{-1}	1	4964	1871.8	37.7	NA	NA	0.5	0.0	3091.7	62.3
218 mg kg^{-1}	2	4964	1649.1	33.2	NA	NA	5.8	0.1	3309.1	66.7
218 mg kg^{-1}	3	4964	2320.8	46.8	NA	NA	5.9	0.1	2637.3	53.1
Mean ± SD		4964 ± 397	1947.2±342.1	39.2 ± 6.9			4.1 ± 3.1	0.1±0.1	3012.7±342.8	60.7 ± 6.9
***S. nutans* vegetated**										
73 mg kg^{-1}	1	1770	0.0	0.0	0.0	0.0	0.5	0.0	1769.5	100.0
73 mg kg^{-1}	2	1770	122.7	6.9	2.5	0.1	0.2	0.0	1644.6	92.9
73 mg kg^{-1}	3	1770	50.2	2.8	0.0	0.0	1.0	0.1	1718.8	97.1
Mean ± SD		1770 ± 527	57.6 ± 61.7	3.3 ± 3.5	0.8 ± 1.4	0.0±0.1	0.6 ± 0.4	0.0±0.0	1711.0 ±62.8	96.7 ±3.6
146 mg kg^{-1}	1	3633	275.4	7.6	10.4	0.3	4.3	0.1	3342.9	92.0
146 mg kg^{-1}	2	3633	178.9	4.9	10.4	0.3	5.1	0.1	3438.6	94.6
146 mg kg^{-1}	3	3633	1159.6	31.9	0.7	0.7	2.5	0.1	2470.2	67.3
Mean ± SD		3633 ± 539	538.0 ± 540.5	14.8 ±14.9	7.2 ± 5.6	0.4±0.2	4.0 ± 1.3	0.1±0.0	3083.9±533.6	84.7 ± 15.1
218 mg kg^{-1}	1	4964	1469.2	29.6	13.1	0.3	0.3	0.0	3481.4	70.1
218 mg kg^{-1}	2	4964	2063.9	41.6	24.7	0.5	17.0	0.3	2858.4	57.6
218 mg kg^{-1}	3	4964	1656.2	33.4	5.3	0.7	12.9	0.3	3289.6	65.7
Mean ± SD		4964 ± 397	1729.8±304.1	34.8 ± 6.1	14.4 ± 9.8	0.5±0.2	10.1 ± 8.7	0.2±0.2	3209.8±319.1	64.5 ± 6.4

Table 16. RDX mass balances of soil-plant systems, in mg per core and % of initial extractable comp-B parent compound (RDX) per core. Measured values, means and standard deviations are shown (N=3). NA=not applicable.

Comp-B Exposure	Block	In. Soil-RDX (mg core^{-1})	Final Soil-RDX		Final Plant-RDX		Leach-RDX		RDX-loss Other Processes	
			(mg core^{-1})	(%)	(mg core^{-1})	(%)	(mg core^{-1})	(%)	(mg core^{-1})	(%)
Bare										
73 mg kg^{-1}	1	2293	849.5	37.0	NA	NA	0.9	0.0	1442.6	62.9
73 mg kg^{-1}	2	2293	1388.5	60.6	NA	NA	8.1	0.4	896.4	39.1
73 mg kg^{-1}	3	2293	1482.1	64.6	NA	NA	8.4	0.4	802.5	35.0
Mean ± SD		2293 ± 340	1240.0 ± 341.1	54.1 ± 14.9			5.8 ± 4.3	0.3 ± 0.2	1047.2 ± 345.7	45.7 ± 15.1
146 mg kg^{-1}	1	4944	813.0	16.4	NA	NA	3.0	0.1	4128.0	83.5
146 mg kg^{-1}	2	4944	1939.3	39.2	NA	NA	5.2	0.1	2999.5	60.7
146 mg kg^{-1}	3	4944	3612.1	73.1	NA	NA	8.7	0.2	1323.2	26.8
Mean ± SD		4944 ± 1187	2121.5 ±1408.4	42.9 ± 28.5			5.6 ± 2.9	0.1 ± 0.1	2816.9 ±1411.3	57.0 ± 28.5
218 mg kg^{-1}	1	6445	3353.5	52.0	NA	NA	5.6	0.1	3085.9	47.9
218 mg kg^{-1}	2	6445	3653.6	56.7	NA	NA	18.0	0.3	2773.4	43.0
218 mg kg^{-1}	3	6445	5801.2	90.0	NA	NA	9.2	0.1	634.6	9.8
Mean ± SD		6445 ± 909	4269.4 ±1335.0	66.2 ± 20.7			10.9 ± 6.4	0.2 ± 0.1	2164.6 ±1334.2	33.6 ± 20.7
S. nutans vegetated										
73 mg kg^{-1}	1	2293	1118.9	48.8	110.1	4.8	7.5	0.3	1056.5	46.1
73 mg kg^{-1}	2	2293	1089.3	47.5	104.7	4.6	3.3	0.1	1095.7	47.8
73 mg kg^{-1}	3	2293	954.1	41.6	138.4	6.0	14.7	0.6	1185.8	51.7
Mean ± SD		2293 ± 340	1054.1± 87.9	46.0 ± 3.8	117.7 ± 18.1	5.1 ±0.8	8.5 ± 5.8	0.4 ± 0.3	1112.7 ± 66.3	48.5 ± 2.9
146 mg kg^{-1}	1	4944	1624.0	32.8	120.0	2.4	22.4	0.5	3177.6	64.3
146 mg kg^{-1}	2	4944	2379.8	48.1	61.8	1.3	12.6	0.3	2489.8	50.4
146 mg kg^{-1}	3	4944	3788.3	76.6	95.6	1.9	12.3	0.2	1047.8	21.2
Mean ± SD		4944 ± 1187	2597.4 ±1098.4	52.5 ± 22.2	92.5 ± 29.2	1.9 ±0.6	15.8 ± 5.8	0.3 ± 0.1	2238.4 ±1086.9	45.3 ± 22.0
218 mg kg^{-1}	1	6445	4435.8	68.8	101.8	1.6	7.0	0.1	1900.4	29.5
218 mg kg^{-1}	2	6445	5395.7	83.7	83.1	1.3	24.6	0.4	941.6	14.6
218 mg kg^{-1}	3	6445	5432.9	84.3	60.6	0.9	19.2	0.3	932.3	14.5
Mean ± SD		6445 ± 909	5088.1 ± 565.2	78.9 ±8.8	81.8 ± 20.6	1.3 ±0.3	16.9 ± 9.0	0.3 ± 0.1	1258.1 ± 556.3	19.5 ± 8.6

Table 17. Stable isotopic ratios, elemental nitrogen, and C/N ratios in selected soil-plant systems amended with 73 mg kg^{-1} comp-B, with and without additional amendments of ^{15}N-labeled RDX. Mean values and standard deviations are shown (N=3). Based on these data the contents of elemental N, ^{15}N, the contributions of ^{15}N and ^{15}N: RDX-N ratios were calculated. NA= not applicable.

Matrix	$\delta^{15}N$ (‰)	Elemental N (%)	C/N	Elemental N (mg $core^{-1}$)	^{15}N (mg $core^{-1}$)	^{15}N (% initial)	Measured RDX (mg $core^{-1}$)[1]	^{15}N: RDX-N
^{15}N amended (BLOCK 3)								
Initial								
Amendment,200-g	23829 ± 12089	1.01 ± 0.41	1.2 ± 0.3	2020	184	NA		
Exposed								
Bare								
Soil, top layer	562 ± 30	0.03 ± 0.00	14.8 ± 1.0	3723	21	11.59	227.63	9.36
Soil, mid layer	839 ± 7	0.02 ± 0.00	14.7 ± 0.3	2482	17	9.10	264.22	6.33
Soil, bottom layer	894 ± 65	0.02 ± 0.00	17.2 ± 2.1	10140	70	38.29	628.61	11.19
Soil, total column				16345	108	58.99	1120.47	9.67
***S. nutans* vegetated**								
Soil, top layer	1635 ± 18	0.03 ± 0.00	12.7 ± 0.7	3723	36	19.56	535.40	6.71
Soil, mid layer	1228 ± 61	0.02 ± 0.00	13.6 ± 0.3	2482	20	11.02	45.74	44.28
Soil, bottom layer	736 ± 45	0.02 ± 0.00	16.9 ± 1.2	10140	64	35.10	140.16	46.01
Soil, total column				16345	121	65.68	721.30	16.73
Plant shoots	17052 ± 1744	1.00 ± 0.02	43.9 ± 0.8	336	22	12.09	91.70	24.21
Plant roots	5567 ± 328	1.31 ± 0.04	29.7 ± 1.3	126	3	1.66	13.00	23.39
Whole plants				462	25	13.74	104.70	24.13

Note: [1]RDX is composed of 75.6% N.

.

The concentrations of TNT and RDX, and their known degradation compounds, were also tested for significance of the effect of vegetative cover per exposure level (73, 146, and 218 mg kg^{-1} comp-B, respectively) and per soil layer (top, mid, and bottom, respectively), but no significant effect of vegetative cover was identified.

Explosives mass balances of lysimeter systems

The explosives mass balances of TNT and RDX were determined.

Final TNT concentrations in vegetated soils were consistently less than in bare soils. TNT loss was attributed largely to loss by other processes (LOP) than accumulation in plants or leaching. These other processes can be bioremediation, plant-assisted or not plant-assisted, complexation with plant material and soil components leading to non-extractability, and photolysis, with the latter being limited to the soil surface only. TNT LOP relatively decreased with increasing comp-B exposure from 96.2% to 64.2% of the TNT extracted from the amendments in vegetated units, and from 89.3% to 60.7% in bare units (Table 15, Figure 19). However, LOP increased when expressed in milligrams per soil core, from 1702.9 mg to 3185.8 mg TNT per core in vegetated units and from 1581.0 to 3012.7 mg TNT per core in bare units (Table 15). TNT accumulation in plant tissues was low, ranging from 0.3 to 0.8%, or 6.2 to 38.4 mg per core (Table 15, Figure 19). The total amount of TNT degradation compounds accumulated in the plants, including 2ADNT and 4ADNT, exceeded that of TNT by a factor of 2.6 (Table A4), but this enhanced the plant containment of TNT and degradation compounds only to a maximum of 80.6 mg per core. TNT leaching was low. In vegetated units, it ranged from 0.1 to 0.2% or 3.3 to 10.1 mg per core (Table 15, Figure 19). In bare units, it ranged from 0 to 0.1% or 1.1 to 4.1 mg per core. The total amount of TNT degradation compounds leached, including 2ADNT, 4ADNT, and 24DANT, was usually on the order of 10% or less of the leached TNT (Figure 15), and was, therefore, not included in the mass balance calculations.

Final RDX concentrations in vegetated soils were less than in bare soils in the units that received the least comp-B amendment, but were greater in the units that received the two greater comp-B amendments (Table 16, Figure 19). RDX loss was attributed largely to LOP rather than to accumulation in plants or leaching. RDX LOP relatively decreased with increasing comp-B exposure from 47.7% to 19.5% of the RDX extracted from the amendments in vegetated units, and from 45.7% to 33.6% in bare units

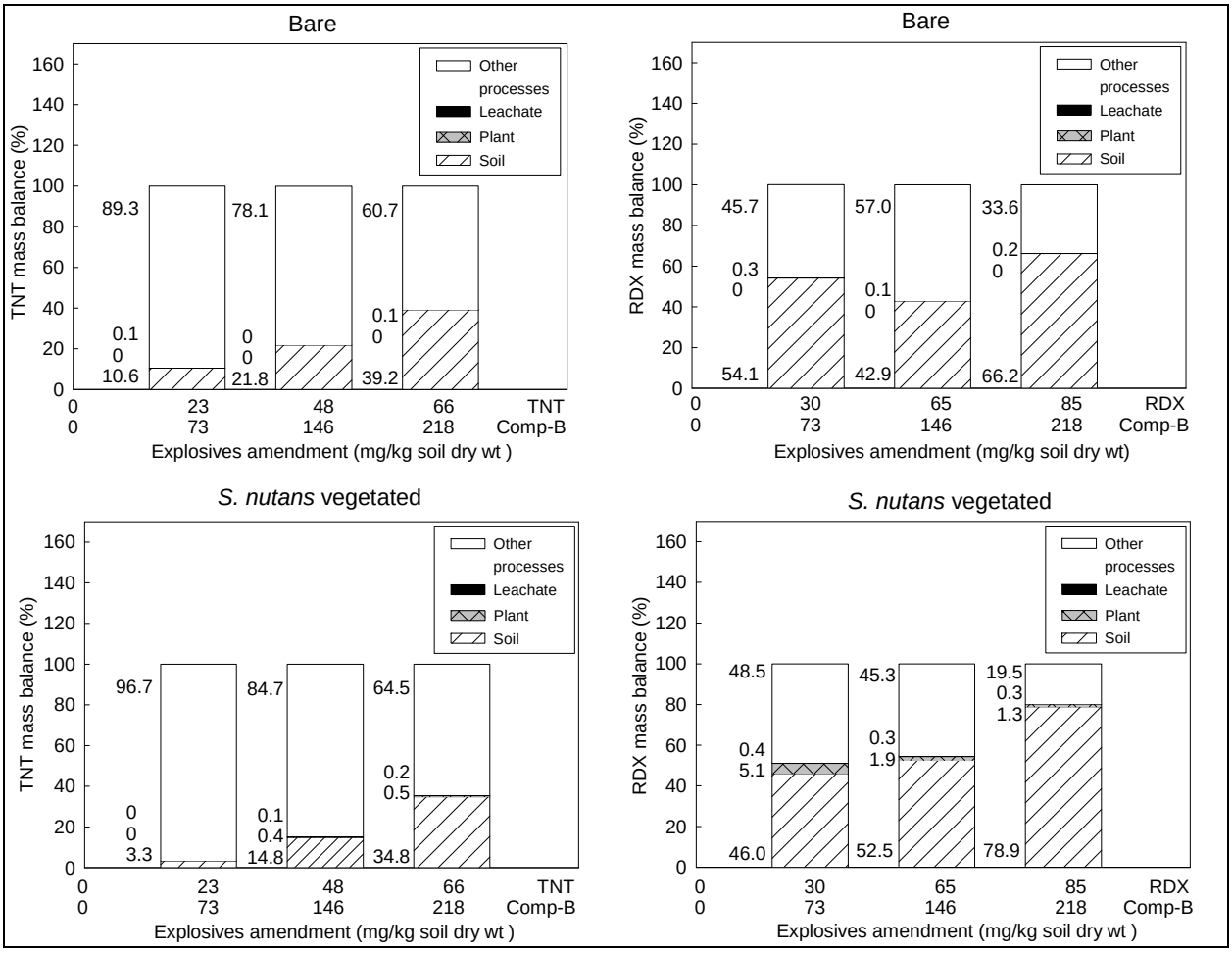

Figure 19. TNT and RDX mass balances of bare and *S. nutans* vegetated soil cores after 92 days of exposure to comp-B. Exposure expressed as amendment with comp-B and its components of interest for this study, TNT and RDX. Numbers left of bars refer to soil, plant, leachate, and other processes (bottom to top).

(Table 16, Figure 19). However, LOP was greatest when expressed per mg soil core in the units that received the 146 mg kg^{-1} comp-B treatment, being 2238.4 mg RDX per core when vegetated and 2816.9 mg RDX per core when bare (Table 16). RDX accumulation in plant tissues was low, ranging from 1.3 to 5.1%, or 81.8 to 117.7 mg per core (Table 16, Figure 19). The total amount of RDX degradation compounds accumulated in the plants, including MNX, was less than 1% of RDX accumulated (Table A4). RDX leaching was low. In vegetated units, it ranged from 0.3 to 1.2% or 15.8 to 27.4 mg per core (Table 16, Figure 19). In bare units, it ranged from 0.1 to 0.3% or 5.6 to 10.9 mg per core. The total amount of RDX degradation compounds leached, including MNX and DNX, was low compared to that of RDX ($\leq$10%; Figure 17), and was, therefore, not included in the mass balance calculations.

Stable isotopic ratios as parameters for tracking mass balances and fate of RDX in lysimeter systems

Amendment of the lysimeters with the lowest comp-B concentration (of 73 mg kg^{-1}) with ^{15}N-RDX resulted in a lower overall enrichment of 7.37 % than targeted (at 22%; Table 17). Overall enrichment (E) was determined as follows.

$$E = \frac{(^{15}N\ of\ 200-g\ ^{15}N-amendment) + (^{15}N\ of\ 75{,}319\ g\ remaining\ core)}{(^{15}N\ of\ 75{,}519\ g)} x\,100(in\,\%) \quad (3)$$

In which

^{15}N of 200-g ^{15}N-amendment = 184 mg core^{-1} (Table 17; measured in Block 3)

^{15}N of 75,319 g remaining core = 7 x (24g4) mg core^{-1} (measured in Block 2)

^{15}N of 75,519 g core = 7 x (24g4) mg core^{-1} (measured in Block 2)

For the mass balance estimates the total quantity of ^{15}N amended to each selected core was calculated at 184 mg core^{-1} (Table 17). The ^{15}N contents of the bare and vegetated soil-plant systems were also calculated and compared with the amended amounts. Results indicated that 58.9% of ^{15}N-amended was recovered in the soil cores of the bare units, versus 65.6% in the soil cores and 13.7% in the plants of the vegetated units (Table 17). These values were compared to the chemical mass balances based on measured RDX values (73 mg kg^{-1} comp-B exposure, Block 3; Table 16). The ^{15}N-mass balance values were slightly less than the chemical mass balance estimates for the bare units (soil, 93% of chemical), but exceeded the chemical mass balance estimates for vegetated units considerably (soil, 158%; plants, 228% of chemical). The greater incorporation of ^{15}N than of RDX in the soil and plants of the vegetated units was attributed to the incorporation of RDX metabolites generated by the increased microbial community and activity, stimulated by exuded plant compounds.

The ^{15}N isotope distribution increased from top to bottom in the exposed soil cores of Block 3 (Table 17). This pattern coincided with the measured RDX distribution in the soil of the same non-vegetated unit, but was the

reverse of the measured RDX distribution in the soil of the vegetated unit (Table 17; values from Tables A4 and A8). The ratio between the ^{15}N content and the measured-RDX-N content was calculated for the soil layers and plants. This ratio increased from top to bottom in the exposed soil cores and was overall greater in the vegetated soil core (16.73%) than in the non-vegetated soil core (9.67%). This ratio was 24.13% in the plants (Table 17). A ratio greater than established with the amendment (7.37%) may indicate the presence of relatively more ^{15}N than RDX, generated by degradation of RDX with RDX transformed into metabolites used by soil microorganisms and plants and to RDX transformation within the plants themselves. Degradation of the amended RDX to nitrogenous compounds may have alleviated the tentative N limitation in the nutrient-poor soil.

Bioavailability of TNT and RDX: concentrations of SPME- and root-associated compounds

An SPME approach was used to provide a biomimetic, non-specific, predictive assessment of the bioavailabilities of TNT and RDX to plants in this experiment. Non-specific bioavailability of explosives was determined to evaluate a tentative relationship between this assay and the specific bioavailabilities to *S. nutans* derived from this plant species root concentrations of TNT and RDX. For this comparison the concentrations of TNT and RDX in milligram compound per kilogram root-dry weight were recalculated per milliliter root dry weight using the measured root length and diameter presented in Table 5. The SPME explosives concentrations were low, and ranged from 0.3 to 2.5 mg TNT mL^{-1} SPME and 1.0 to 1.3 mg RDX mL^{-1} SPME (Table 18). However, the SPME explosives concentrations were far greater than those extracted from the roots and expressed on mg ml^{-1} root-basis, i.e., for TNT a factor of 0.1×10^6 to 1×10^6, and for RDX a factor of 0.1×10^7 to 247 (Table 18).

The SPME TNT level was significantly affected by comp-B exposure over the entire exposure period, but that of SPME RDX was not (Table 18, Figure 20). The SPME TNT and SPME RDX levels were not significantly affected by exposure period of the lysimeter units from which the leachates were collected (Table 18), just as the TNT and RDX concentrations in the leachates of the vegetated units. The effect of the interaction term 'Comp-B exposure' x 'Exposure period' was not significant. SPME explosives levels did not exhibit a statistically identifiable relationship with root explosives levels. Relationships explored included ANOVA and various regression models applied to the measured and to the log-transformed data.

Table 18. TNT and RDX concentrations extractable from SPMEs and roots of *S. nutans* in response to exposure to comp-B. SPMEs were exposed to leachates and roots were harvested from the lysimeter systems at monthly intervals. Mean values and standard deviations are shown (N=3). ANOVA[1] results are listed.

Comp-B	SPME		Root				
	(mg mL^{-1} SPME)		(mL g^{-1} dry wt) x 10^3	(mg kg^{-1} dry wt)		(mg mL^{-1} root) x 10^{-7}	
Exposure, days	TNT	RDX	Volume	TNT	RDX	TNT	RDX
73 mg kg^{-1}, 28 d	0.3 ± 0.3	1.2 ± 0.4	955	300.5 ± 98.7	1144.6 ± 200.7	3.1 ± 1.3	12.0 ± 2.1
73 mg kg^{-1}, 63 d	0.4 ± 0.0	1.2 ± 0.1	955	120.9 ± 85.9	1343.7 ± 830.7	1.3 ± 0.9	14.1 ± 8.7
73 mg kg^{-1}, 92 d	0.5 ± 0.0	1.0 ± 0.1	955	220.0 ± 381.1	3461.6 ± 1495.7	2.3 ± 4.0	36.2 ±15.7
146 mg kg^{-1}, 28 d	1.8 ± 1.3	1.2 ± 0.3	1031	1081.0 ± 279.7	1583.7 ± 460.3	10.5 ± 2.7	15.4 ± 4.5
146 mg kg^{-1}, 63 d	0.3 ± 0.0	1.1 ± 0.1	1031	507.7 ± 162.7	1854.2 ± 296.0	4.9 ± 1.6	18.0 ± 2.9
146 mg kg^{-1}, 92 d	1.3 ± 0.8	1.1 ± 0.2	1031	667.7 ± 498.3	3314.2 ± 2640.7	6.5 ± 4.8	32.1 ± 25.6
218 mg kg^{-1}, 28 d	2.5 ± 3.6	1.3 ± 0.4	356	1290.0 ± 191.2	1778.2 ± 570.0	36.2 ± 5.4	50.0 ± 16.0
218 mg kg^{-1}, 63 d	2.0 ± 2.7	1.3 ± 0.2	356	1337.7 ± 69.8	2721.7 ± 1291.7	37.6 ± 2.0	76.5 ± 36.3
218 mg kg^{-1}, 92 d	2.5 ± 1.8	1.3 ± 0.3	356	2566.9 ± 1709.6	5754.3 ± 3607.3	72.1 ± 48.0	162.0 ±101.0
ANOVA[1]							
Factor				MS	F-ratio	p-value	
Comp-B exposure – SPME-TNT				7.96	352	0.049	
Comp-B exposure – SPME-RDX				0.11	2.40	0.117	
Exposure period – SPME-TNT				1.09	0.48	0.625	
Exposure period – SPME-RDX				0.01	0.38	0.690	

[1] ANOVA results of SPME data, using 'Comp-B exposure' and 'Exposure period' as factors, respectively, and 'Block' as covariate. Underlining marks a statistically significant effect.

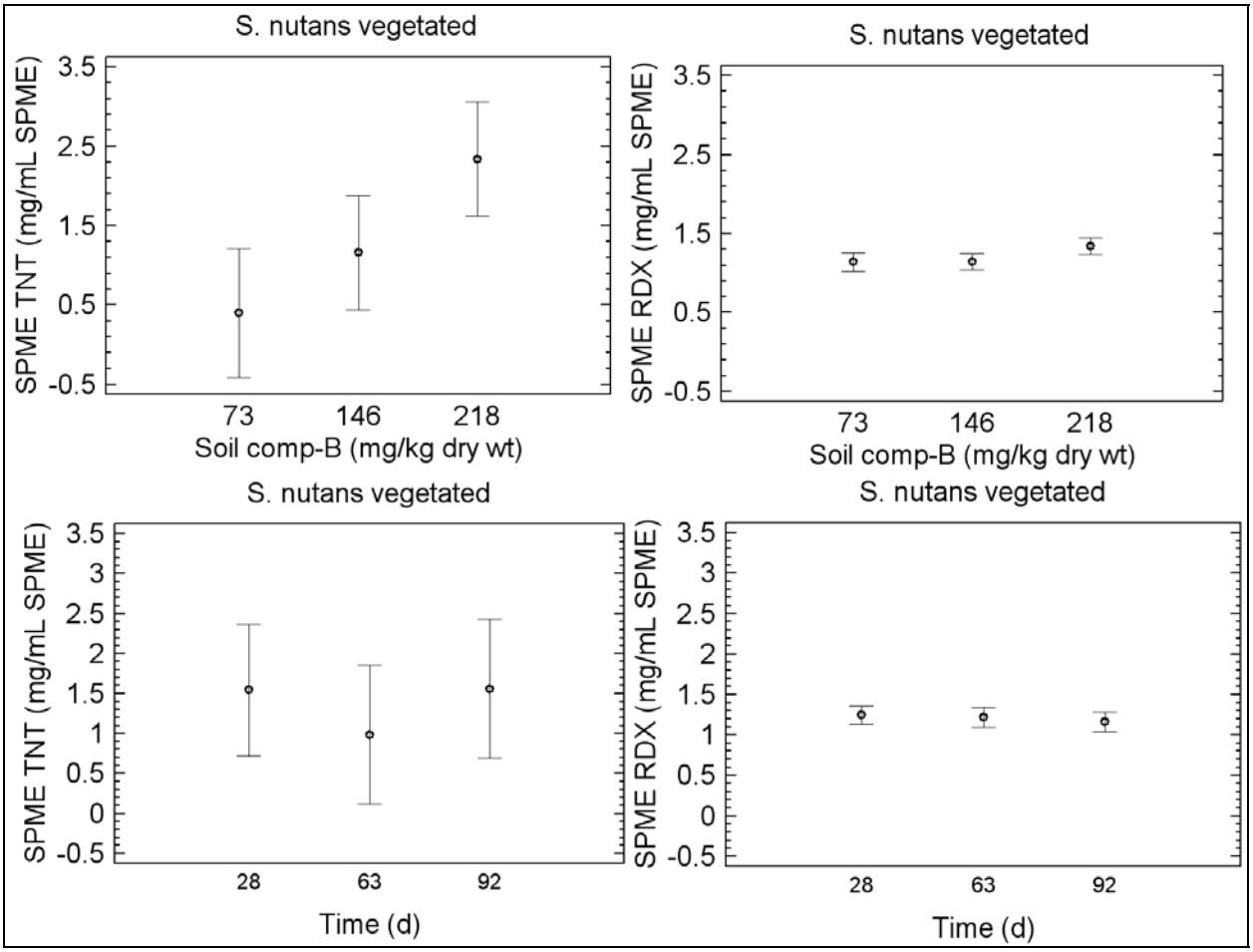

Figure 20. TNT and RDX concentrations extractable from SPMEs exposed to leachates collected from *S. nutans* vegetated units exposed at monthly intervals to comp-B. Mean values and standard errors of the mean, generated by ANOVA. Upper, using 'Comp-B exposure' as factor, and 'Block' as covariate. Lower, using 'Exposure period' as factor, and 'Block' as covariate.

Microbial communities in the surface layers of vegetated and bare soil cores exposed to comp-B treatment

Principal component analysis revealed two outlier samples, i.e., B1BA-T32 and B1BA-T21 – both bare soil samples (BA; Figure 21). After removal of these samples, results of the new exploratory analysis suggested that for both the bare soil and the *S. nutans* vegetated soil (SN) the greater comp-B concentration, 218 mg kg^{-1} (T3, with replicates 1,2 and 3), had an effect on the microbial community composition. Total microbial biomass appeared to be a significant variable with the 218 mg kg^{-1} samples showing a mean biomass of 76 $\pm$ 33 and the rest of the samples a mean of 171 $\pm$ 58 pmole g^{-1} soil-dry wt (outliers removed).

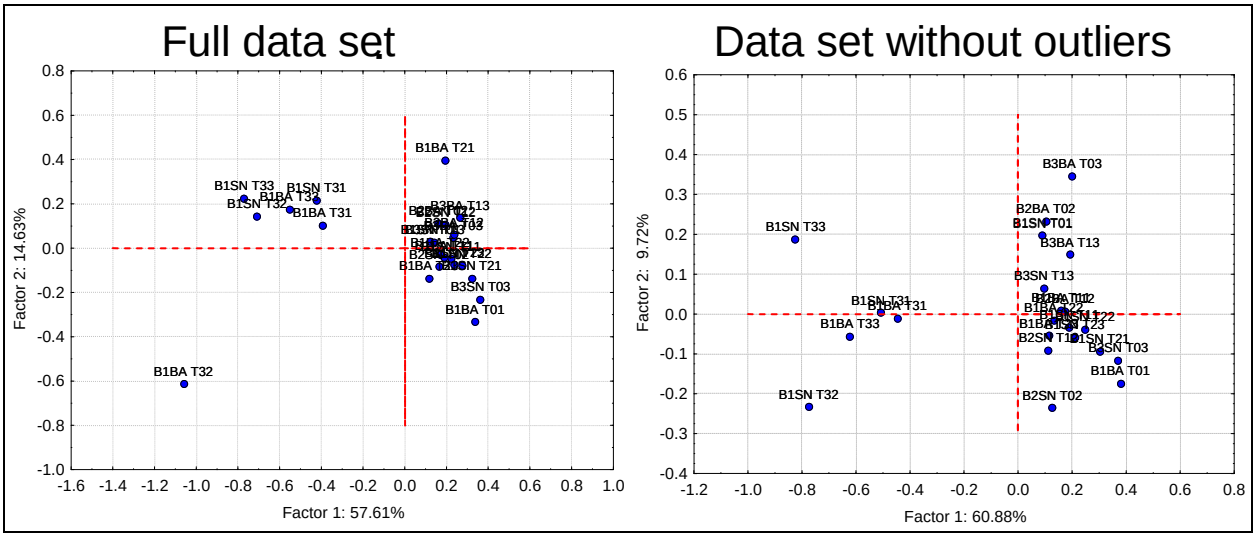

Figure 21. Principal component analysis of PLFA profiles of the microbial communities in the top layers of bare and vegetated soils exposed to comp-B and controls. Full data set (left) and data set without outliers (B1BA T32 and B1BA T21; right).

Within total microbial biomass, four functional groups were distinguished based on their PLFAME biomarkers: normal saturates (NS; ubiquitous bacteria), terminally branched saturates (TBS; Gram-positive bacteria), mono-unsaturates (M; Gram-negative bacteria), and mid-chain methyl-branched saturates (MBS; Actinomycetes). In the bare soils, the 218 mg kg^{-1} samples showed increased mole percentages of the long-chain saturates 20:0, 22:0 and 24:0 and decreased percentages of the terminally branched saturates (specifically i16:0 and i17:0), the normal saturates 16:0 and 17:0 and the mid-chain methyl branched saturate 10me16:0 relative to the controls (Table 19). In other words, every functional component of the observed microbial community decreased except for the Gram-negatives (i.e., the mono-unsaturates). In the vegetated soils, the 218 mg kg^{-1} samples showed an increase in the long chain saturated PLFAME and similar declines in the mole percentages of terminally branched and saturated PLFAME. However, these samples also showed a small increase in a single Gram-negative biomarker, cy 19:0.

In the vegetated soils a presumed hysteresis effect of the explosives at 146 mg kg^{-1} was observed. At the latter level, biomass and Gram-positive biomarkers increased significantly, while Gram-negative biomarkers increased, but not significantly (Figure 22).

Table 19. Post hoc Fisher's Least Significant Difference (α=0.05) procedure results of microbial biomass and biomarkers (outliers removed). Values that are followed by the same letter are not significantly different.

Biomass/ Biomarker	Bare				*S. nutans* Vegetated			
	Comp-B Exposure (mg kg^{-1} dry wt)				Comp-B Exposure (mg kg^{-1} dry wt)			
	0	73	146	218	0	73	146	218
TBS[a]	B	B	B	A	B	B	B	A
16:0	B	B	B	A	B	B	B	A
17:0	B	B	AB	A	AB	B	AB	A
18:0					A	B	A	C
20:0	A	A	A	B	C	BC	A	D
22:0	A	A	A	B	A	A	A	B
24:0	A	A	A	B	A	A	A	B
i16:0	B	B	B	A	B	B	B	A
i17:0	B	B	B	A	AB	BC	C	A
a17:0					B	B	B	A
10me16:0	B	B	B	A	B	B	B	A
12me18:0					A	B	A	B
cy19:0					A	AB	A	B

[a] TBS = terminally branched saturates, or Gram-positive bacteria.

When comparing the microbial community compositions of the bare and vegetated soils, only one fatty acid, 16:0, showed significant differences at the 0.05 significance level at the 146 and 218 mg kg^{-1} comp-B treatment levels. Dropping the significance level to 0.01 produced only a few more significant differences (Table 20). Although it appears that the bare and vegetated soils responded differently to the explosives treatment levels, the microbial community response was subtle and clouded by a high variability between the replicate lysimeters.

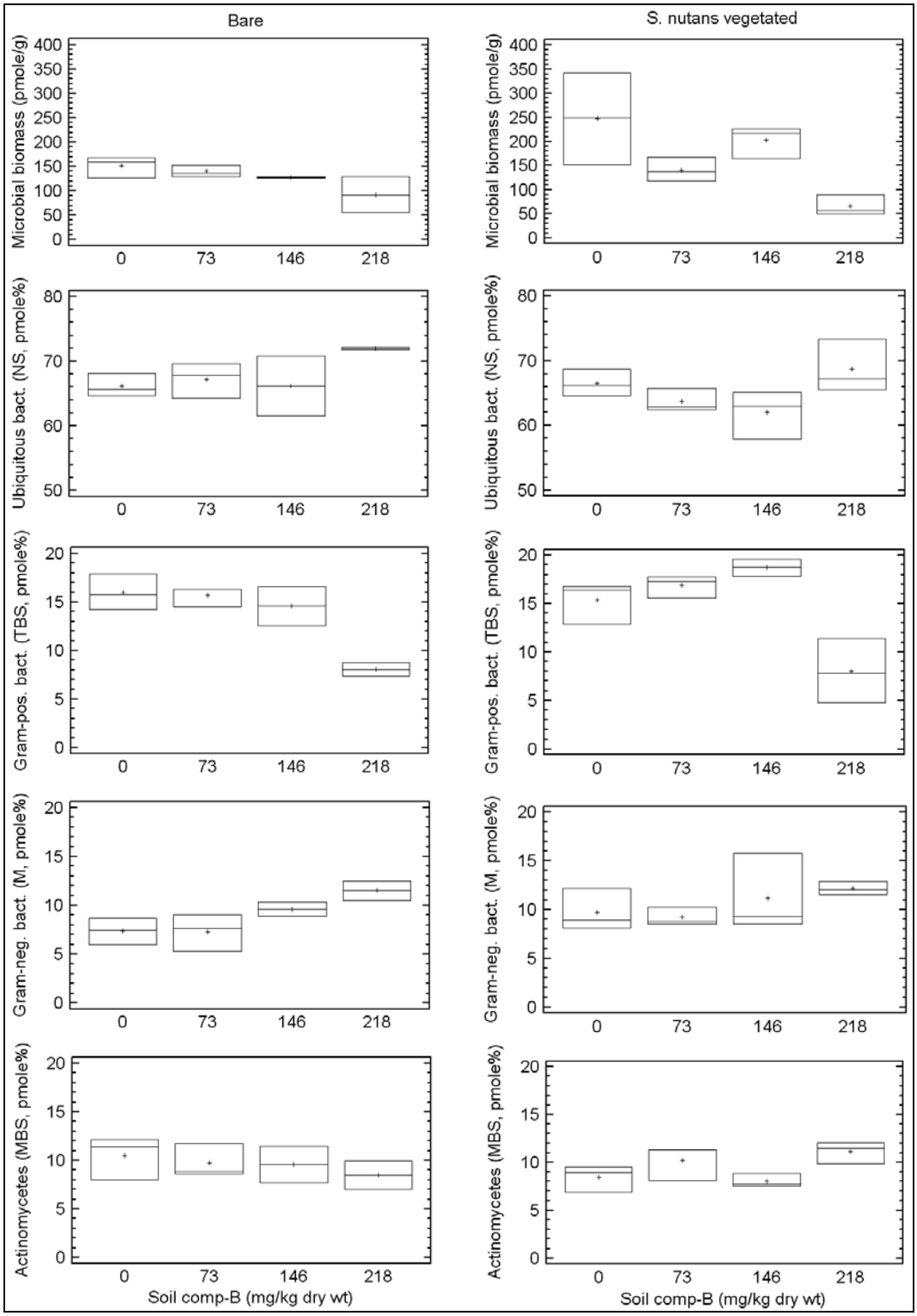

Figure 22. Microbial biomass and community composition in the top layers of bare and vegetated soils exposed to comp-B and controls. Box-and-Whisker plots, with means marked by a cross. Biomass in unexposed soil was 82.6 ± 17.8 pmole/g. 1 pmole PLFA is equivalent to 2.5 x 10^4 cells.

Table 20. Pair comparison of microbial biomass and biomarkers using Student t test (α=0.01; outliers removed). Values that are followed by the same letter are not significantly different.

Biomass/ Biomarker	Comp-B Exposure (mg kg^{-1} dry wt)							
	0		73		146		218	
	Bare	Veg[a].	Bare	Veg[a].	Bare	Veg[a].	Bare	Veg[a].
Biomass					A	B		
16:0					A	B	A	B
17:0	A	B						
22:0			A	B	A	B		
i15:0					B	A		
i17:0	A	B						
12me18:0			B	A				

[a] Veg.= *S. nutans* vegetated.

4 Discussion

Phytoremediation of comp-B derived explosives in lysimeter systems

In the present study two herbaceous plant species were evaluated for potential application in phytoremediation systems on training ranges. The grass *Sorghastrum nutans* showed the ability to persist, produce considerable biomass on the range soil, and to tolerate comp-B exposure up to a level of 218 mg kg^{-1} soil. The forb *Amaranthus retroflexus* germinated, but was unable to persist on the range soil. Failure of the latter species to grow was attributed to low nutrient availability (particularly nitrogen), since in the previous pot experiment in which tolerance to explosives was tested under fertilized and field mimicking environmental conditions substantial biomass was produced by *A. retroflexus* (Best et al. 2008).

Mass balance results indicated that the soil cores contained less TNT at the end of the three-month exposure period when vegetated with *S. nutans* than unvegetated when treated with comp-B up to a level of 218 mg kg^{-1}. Soil cores also contained less RDX when vegetated than unvegetated, but only when treated with comp-B at a level of 73 mg kg^{-1}.

Most of the comp-B derived TNT and RDX remained contained in the lysimeter units, since losses with leaching were extremely small (maximally 10.1 mg TNT per system, 92.1 mg m^{-2} soil, or 0.2%; and 16.9 mg RDX, 154.1mg m^{-2} soil, or 0.3% in a 92-day period; Tables 16 and 17) and volatilization was likely negligible since neither TNT nor RDX are volatile. Accumulation and potential subsequent loss with plant materials by harvesting was also small (maximally 14.4 mg TNT per system, 131.3 mg m^{-2} soil, or 0.5%; and 117.7 mg RDX, 1073.4 mg m^{-2} soil, or 5.1% in a 92-day period; Tables 16 and 17), leaving the greatest explosives losses from the lysimeter systems (up to 89.3% of TNT and 57.0% of RDX) to processes other than accumulation in plants or leaching. These other processes can be bioremediation, plant-assisted or not plant-assisted, complexation with plant material and soil components leading to non-extractability, and photolysis, with the latter being limited to the soil surface only.

Uptake and accumulation of TNT and RDX in plants

Uptake by roots and subsequent within plant translocation are important processes in the transfer of non-volatile organic chemicals from soil to various plant parts, while the transpiration stream constitutes the suction force that pulls the water column up. The importance of transpiration for the absorption and translocation of organic chemicals in plants is illustrated by the equation developed by Paterson et al. (1994), i.e.: U = TSCF x T x C. In this equation, U is the rate of chemical assimilation (mg d^{-1}); T is the rate of plant transpiration (d^{-1}); C is the chemical concentration in the water phase of the soil (mg L^{-1}); TSCF is the transpiration stream concentration factor (dimensionless), showing the ratio between the chemicals' concentration in the liquid of the transpiration stream and its concentration in the environment.

Several regression models exist that simplify plant uptake and express this process in terms of chemical properties such as the logarithm of the compound's octanol-water partition coefficient, log K_{OW}. Based on results of organic chemical uptake studies in hydroponics, Briggs et al. (1982) related the log K_{OW} of organic compounds to the transpiration stream concentration factor (TSCF). The TSCF is calculated as the concentration in the transpiration stream divided by the bulk solution concentration in contact with the root tissues. TSCF values determined for organic compounds reach a maximum of 0.8 at a corresponding log K_{OW} of 1.8. The interaction of the compound and the root surface is a determining process in compound translocation, since the chemical must pass the symplast of the endodermis to be translocated from the roots (Trapp et al. 1994). Compounds with lower hydrophobicity, log K_{OW}<1.8, will not pass through the lipid membranes associated with the epidermis layers of the roots. In contrast, more hydrophobic compounds, log K_{OW} >1.8, can enter the root tissues but not the xylem for translocation from the roots to the shoots. These compounds are believed to become bound in the mucigel associated with the root surface and to the lipid membranes of the root epidermis (Clarkson 1991). As a consequence, binding or exclusion at the root interface decreases translocation and leads to lower TSCF values at log K_{OW} values greater or less than 1.8. A model similar to that of Briggs et al. (1982) was developed subsequently by Burken and Schnoor (1998), in which log TSCF = 0.756 exp{-(log K_{OW} – 2.50)2/2.58}. TNT is a hydrophobic compound with a log K_{OW} of 1.86 and RDX a hydrophilic compound with a log K_{OW} of 0.86 (Walsh et al. 1995). The model of Burken and Schnoor (1998) fitted experimental results from hydroponic studies

well, indicating TSCFs of 0.46 for TNT (Thompson et al. 1998) and 0.13 for RDX (Burken and Schnoor 1998) in hybrid poplar. TSCF factors derived from uptake studies from soil have not been published.

Accumulation of TNT and RDX in the plants of the present study was considerable. Total explosives levels accounted for maximally 0.5% of dry wt in shoots and 0.6% in roots (Table 6). By scaling the mg per core values up to mg per m^2 surface area (by a factor of 9.115; Tables 15 and 16), it was found that accumulation in plants ranged from 56.5 to 350.0 mg TNT m^{-2}, and from 730.1 to 1005.4 mg RDX m^{-2}. TSCF factors were estimated for the greatest comp-B exposures using the Paterson et al. (1994) equation. For these estimates, the latter explosives accumulation values (mg m^{-2}), the measured (evapo) transpiration rate of 2 L m^{-2} d^{-1} (Table 2), and the mean levels of TNT (+ 2ADNT and 4ADNT) and RDX (+MNX) in the leachates were used as measures for levels in soil solution (0.003 mg L^{-1}). Following this approach, TSCFs of 633 for TNT and of 1432 for RDX were found. These values greatly exceed the maximum published TSCF value of 0.8, which was attributed to the far lower explosives levels in the collected leachates than in the upper soil layer where the comp-B amendments were applied and the plants were rooted. Theoretical TNT and RDX levels in the soil water of the upper soil layers were calculated by dividing the initial mean extractable TNT and RDX levels of 66 mg TNT kg^{-1} and 85 mg RDX kg^{-1} (Table 4) by the soil moisture content of 0.36 L (36%), generating 183 mg TNT L^{-1} and 236 mg RDX L^{-1}. For the recalculations of the TSCFs, 100 mg TNT L^{-1} (Monteil-Rivera et al. 2004) and 35 mg RDX L^{-1} (Lynch 2002) were used, since these levels represent the solubilities of TNT and RDX at 20 ^oC whereas the theoretical values exceed them. Thus, TSCFs of 0.019 for TNT and of 0.123 for RDX were found in *S. nutans*. These are the first TSCF values for TNT and RDX from soil in plants. The TNT TSCF is far less, while the RDX TSCF is similar to the published TSCF values of 0.46 for TNT (Thompson et al.1998) and 0.13 for RDX (Burken and Schnoor 1998) for poplars in hydroponics. The low TNT TSCF of *S. nutans* may be the mechanism that makes this plant species TNT tolerant.

Sorption and leaching of TNT and RDX in Camp Shelby soil

The sorption coefficients for the Camp Shelby soil of 1.59 L kg^{-1} for TNT and 0.57 L kg^{-1} for RDX indicated a three times lower potential for leaching of TNT than for RDX. The TNT and RDX K_d were similar to those found in surface soil of the Louisiana Army Ammunition Plant (LAAP), Adler soil and Plymouth soil with similar CECs and lower TOC contents

(Table 21; Price et al. 2000; Dontsova et al. 2006). The Camp Shelby soil TNT K_d were far less than those determined in surface soils from AAPs with higher CECs and TOC contents, which could be as high as 6.2 L kg^{-1} (C.B. Price, ERDC Environmental Laboratory unpublished results, 2009). Thus, ranges with more organic soils have a lower leaching potential for TNT and RDX than ranges with low organic matter and clay contents.

Table 21. K_d values obtained when sorbing TNT and RDX onto soils.

Soil	CEC (mmol g^{-1})	TOC (% dry wt)	Sand (% dry wt)	Silt (% dry wt)	Clay (% dry wt)	K_d (L kg^{-1} dry wt)		Reference
						TNT	RDX	
LAAP A	2.5	0.31	89	5	6	1.09	ND	Price et al. 2000
LAAP C	6.6	0.08	77	11	12	1.06	ND	Price et al. 2000
LAAP D	15.5	0.20	27	41	32	1.67	0.286	Price et al. 2000
Adler silt loam	7.83	0.20	11	84	5	2.4	0.48	Dontsova et al. 2006
Plymouth loamy sand	7.05	0.78	87	8	5	1.6	0.65	Dontsova et al. 2006
Adler-Plymouth comparable[a]						1.8 (0.27-4.5)	0.93 (0.16-2.2)	Dontsova et al. 2006
Camp Shelby loamy sand	4.85	1.84	89	6	5	1.59	0.57	This study

Note: ND = not determined

[a] Comparable with respect to CEC and TOC.

Low sorption coefficients for explosives may indicate a considerable potential for leaching. Leaching of TNT and RDX from the 0.5-m cores enclosed in the lysimeter units was usually for TNT 1 mg L^{-1} when vegetated and 0.2 mg L^{-1} unvegetated, and for RDX 2.5 mg L^{-1} when vegetated and 2 mg L^{-1} unvegetated, with elevated levels 7 to 42 days after amendment (Figures 16 and 17). Vegetation may have enhanced the permeability of the soil and decreased explosives sorption to soil particles by rooting on one hand, but decreased explosives in soil by plant uptake and plant-enhanced microbial transformation on the other hand, with the overall effect being that more explosives were leached and less explosives remained in the soil. The explosives levels in the leachates that had passed through a 0.5-m soil column were low, but exceeded the lifetime health advisory for drinking water of 1.0 µg TNT L^{-1} and 2.0 µg RDX L^{-1} (http://www.epa.gov/ost/drinking/standards/dwstandards.pdf). This may indicate that leaching of explosives may form a hazard for shallow water bodies exposed to explosives leachates from ranges.

Bioavailability of comp-B derived TNT and RDX: Comparison of SPME- and root-associated compounds

SPME-associated and root-associated TNT increased significantly with comp-B exposure, while SPME-associated and root-associated RDX did not show a clear relationship with comp-B exposure. However, no relationships between SPME-associated and root-associated TNT and RDX were found. SPME-associated TNT and RDX greatly exceeded root-associated TNT and RDX, i.e., by a factor of 247 to 10^6 (Table 17, Figure 20). These differences may be explained by differences in magnitude of processes involved in the association of explosives with SPMEs and plant roots. Adsorption processes largely determine association of explosives in the leachate to SPMEs, and SPME sorption capacity is far smaller than leachate explosives content. Uptake of soluble compounds and translocation processes determine the association of explosives in the soil solution with plant roots, with uptake largely depending on solubility, log K_{OW} and translocation forming the suction force to pull the solution up. In the present study, the resulting net accumulation of explosives in roots was far less than it might have been when resulting from adsorption alone, indicating that these processes are effective protectors of plant health towards these xenobiotics. Thus, SPMEs are not suitable to predict bioavailability of TNT and RDX in soil water for plants.

Microbial communities in Camp Shelby soil as affected by comp-B derived explosives

Total microbial biomass decreased with increasing comp-B exposure. Biomass was greater in vegetated than unvegetated units, except when exposed to the comp-B level of 218 mg kg^{-1}. The greater microbial biomass in the soil of the vegetated units was attributed largely to plant-leachates, predominated by carbohydrates, which served as a carbon source in the organic matter-poor soil, but nitrogenous compounds resulting from explosives transformation by plants and microbes may have contributed also. Although results of pairwise statistical comparison indicated several differences, no distinct pattern was identified.

Application potential of explosives phytoremediation on a field scale

Although phytoremediation is being applied, the success of phytoremediation projects is not frequently controlled and the applicability and potential for phytoremediation is not assessable (Trapp 2000). Several

projects on various scales in space and time are underway in support of applicability and assessment of phytoremediation potential of explosives-contaminated soil.

The present study focused on herbaceous plants endemic in the United States with the ability to persist on training ranges due to their tolerance towards and ability to degrade TNT and RDX. Of the plants identified, two species were used in this scaled up lysimeter study. Only one of these species persisted, i.e., the grass *S. nutans*, enabling a comparison of remediation of comp-B derived TNT and RDX in vegetated and unvegetated soil cores. A first-order rate equation was fitted to the initial and final soil-TNT and –RDX contents of the soil cores (Tables 15 and 16) after scaling the values up to a surface area of 1 m^2. In this equation $[A] = [A_0] \times e^{-kt}$, [A] is the explosive parent concentration after 92 days of incubation (t_{92}), $[A_0]$ is the initial explosive parent concentration, k is the rate constant and t is time in days. Using this equation, the first order rate constants for the explosives in the vegetated and bare 0.5-m soil columns were calculated. The rate constants generally decreased with increasing comp-B exposure, and ranged in vegetated soils from 0.0092 to 0.0372 d^{-1} for TNT and 0.0026 to 0.0084 d^{-1} for RDX, and in unvegated soils from 0.0102 to 0.0244 d^{-1} for TNT and 0.0045 to 0.0092 d^{-1} for RDX (Table 22). Using the same equation with the calculated rate constants, explosives half-lives were estimated which ranged in vegetated soils from 19 to 60 days for TNT and 82 to 270 days for RDX, and in unvegetated soils from 28 to 68 days for TNT and 75 to 155 days for RDX (Table 22). It must be borne in mind that these half-lives would only occur under the same climatological conditions as in this experiment, being spring and summer temperatures of $\geq$20 ^{o}C and a precipitation regime enabling high soil moisture levels of approximately 36%.

The remediation potential per annum was estimated to enable comparison with other published values pertaining to scaled up and field studies. For this estimate it was assumed that the growth season would be a factor of 1.98 x longer than the experimental period, and that remediation would cease completely outside the growth season. These estimates indicated that the greatest annual remediation potential in vegetated soils was 58.5 g TNT m^{-2} and 42.4 g RDX m^{-2}, and 54.5 g TNT m^{-2} and 51.0 g RDX m^{-2} in unvegetated soils (Table 22), with remediation in vegetated soils exceeding that in bare soils for TNT up to a comp-B level of 218 mg kg^{-1}, and for RDX up to a comp-B level of 73 mg kg^{-1}. In comparison, phytoremediation

potential values for TNT determined in Germany, ranged from 0.8 to 6.0 g m^{-2} for five-year-old trees and 4.2 g m^{-2} for a 45-year old spruce forest (Schoenmuth and Pestemer 2004). The fact that the phyto-remediation potential of herbaceous plants in the United States greatly exceeded that of trees in Germany was attributed to the relatively high temperatures and high soil moisture level of the present experiment. However, both plant groups appear suitable for phytoremediation of soil-based explosives, with herbaceous plants being applied to ranges with an open vegetation structure and trees applied to former military sites. Whether or not the aboveground plant parts may return some of the explosives and/or degradation compounds to the environment upon scenescence (Yoon et al. 2006) is still a potential issue that has to be addressed in scaled up applications.

Table 22. First-order rate constants, half-lives, and remediation potential of explosives in bare and vegetated soil systems.

Comp-B Exposure	First-order Rate Constant (d)		Explosives Half-life (d)		Remediation Potential[a] (g m^{-2} a^{-1})	
	TNT	RDX	TNT	RDX	TNT	RDX
Bare						
73	0.0244	0.0067	28	104	28.6	19.0
146	0.0166	0.0092	42	75	51.4	51.0
218	0.0102	0.0045	68	155	54.5	39.3
S. nutans Vegetated						
73	0.0372	0.0084	19	82	30.9	22.4
146	0.0092	0.0070	33	99	56.0	42.4
218	0.0115	0.0026	60	270	58.5	24.5

[a] Extrapolation core surface area of 0.1097 m^2 to 1 m^2: x 9.12

Extrapolation 92-d exposure period (2 June-2 Sept) to 183-d growth season MS (1 April - 1 October): x 1.98

5 Conclusions

The study described in this report has resulted in the following conclusions.

- Phytoremediation of comp-B derived TNT and RDX was quantified in 0.5-m grass vegetated Camp Shelby soil over a 92-day period.
- Remediation in vegetated soils exceeded that in bare soils for TNT up to a comp-B level of 218 mg kg^{-1}, and for RDX up to a comp-B level of 73 mg kg^{-1}. Thus, phytoremediation can be used as an effective remediation technology in a given range of explosives contamination.
- The greatest annual remediation potential was 58.5 g TNT m^{-2} and 42.4 g RDX m^{-2} in vegetated soils, and 54.5 g TNT m^{-2} and 51.0 g RDX m^{-2} in unvegetated soils.
- Remediation was attributed for a large part to processes other than plant uptake, including bioremediation, either or not plant-assisted, complexation with plant material and soil components leading to non-extractability, and photolysis (limited to the upper soil layer).
- Results of a comparison between ^{15}N-based and chemical-RDX-based mass balances, with ^{15}N derived from uniformly labeled ^{15}N-RDX, indicated greater incorporation of ^{15}N than of RDX in soil and plants of vegetated units than in soil of non-vegetated units. This was attributed to the incorporation of RDX metabolites generated by the increased microbial community biomass and activity, stimulated by exuded plant compounds, and to RDX transformation within the plants themselves. Degradation of the amended RDX to nitrogenous compounds may have alleviated the tentative N limitation in the nutrient-poor soil.
- Sorption coefficients for Camp Shelby soil were low, indicating considerable potential for explosives leaching. These coefficients were three times greater for TNT than RDX.
- Despite the considerable leaching potential derived from the measured sorption coefficients, leaching was very low compared to loss of explosives due to other processes than plant uptake.
- The microbial communities in the upper soil layer showed decreased biomass with increasing comp-B exposures. Community shifts were subtle if at all. However, a presumed hysterisis effect was observed in the vegetated soil at a comp-B exposure of 146 mg kg^{-1} soil.

References

Adamia, G., G. Ghoghoberidze, D. Graves, G. Khatisashvili, G. Kvesitadze, E. Lomidze, D. Ugrekhelidze, and G. Zaalishvili. 2006. Absorption, distribution and transformation of TNT in higher plants. *Ecotoxicology Environmental Safety* 64: 136-145.

Allen, S. E., H. M. Grimshaw, J. A. Parkinson, and C. Quarmby. 1974. *Chemical analysis of ecological materials*. S.E.Allen et al. (eds). Wiley, New York: 21-22.

Best, E. P. H., K. N. Geter, H. E. Tatem, and B. K. Lane. 2006. Effects, transfer, and fate of RDX from aged soil in plants and worms. *Chemosphere* 62: 616-625.

Best, E. P. H., G. Kvesitadze, G. Khatisashvili, and T. Sadunishvili. 2005. Plant processes important for the transformation and degradation of explosives contaminants. *Zeitschrift fuer Naturforschung* 60c: 340-348.

Best, E. P. H., T. Smith, F. L. Hagen, J. Dawson, and A. J. Torrey. 2008. *TNT and RDX phytoremediation capacities of candidate herbaceous plants for phytoremediation of energetics on ranges*. ERDC TR 08-8. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Best, E. P. H., S. L. Sprecher, S. Larson, H. L. Fredrickson, D. F. Bader. 1999. Environmental behavior of explosives in groundwater from the Milan Army Ammunition Plant in aquatic and wetland plant treatments. Uptake and fate of TNT and RDX in plants. *Chemosphere* 39: 2057-2072.

Bhadra, R., R. J. Spanggord, D. G. Wayment, J. B. Hughes, and J. V. Shanks. 1999. Characterization of oxidation products of TNT metabolism in aquatic phytoremediation systems of *Myriophyllum aquaticum*. *Environmental Science and Technology* 33: 3354-3361.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemical Physiology* 37: 911-917.

Brannon, J. M., C. B. Price, C. Hayes, and S. L. Yost. 2002. Aquifer soil cation substitution and adsorption of TNT, RDX, and HMX. *Soil and Sediment Contamination* 11: 327-338.

Bremner, J. M., and C. S. Mulvaney. 1982. In *Methods of soil analysis, Part 2: Chemical and microbiological properties*. 2nd ed., A. L. Page et al., 595-624. Madison, WI: SSSA Book Series 5, ASA, SSSA.

Briggs, G. G., R. H. Bromilow, and A. A. Evans. 1982. Relationships between lipophilicity and root uptake and translocation of non-ionized chemicals by barley. *Pesticide Science* 13: 495-504.

Burken, J. G., J. L. Schnoor. 1998. Predictive relationships for uptake of organic contaminants by hybrid poplar trees. *Environmental Science and Technology* 32: 3379-3385.

Burken, J. G., J. V. Shanks, and P. L. Thompson. 2000. Phytoremediation and plant metabolism of explosives and nitroaromatic compounds. In *Biodegradation of nitroaromatic compounds and explosives*, ed. J. C. Spain, J. B. Hughes, and H. J. Knackmuss, Chapter 10: 239-275. Boca Raton, FL: Lewis Publishers.

Cataldo, D. A., S. D. Harvey, R. J. Fellows, R. M. Bean, and B. D. McVeety. 1989. *An evaluation of the environmental fate and behavior of munitions material (TNT, RDX) in soil and plant systems*. PNL-7370, AD-A223 546, Fort Detrick, MD: U.S. Army Medical Research and Development Command.

Clarkson, D. T. 1991. Root structure and sites of ion uptake. In *Plant roots the hidden half*, ed. Y Waisel, A. Eishel, V. Kafkafi, 415. New York: Marcel-Dekker, Inc.

Conder, J. M., T. W. La Point, G. R. Lotufo, and J. A. Steevens. 2003. Non-destructive, minimal disturbance, direct-burial solid-phase microextraction fiber technique for measuring TNT in sediment. *Environmental Science and Technology* 37: 1625-1632.

Conder, J. M., and T. W. La Point. 2005. Solid-phase microextraction for predicting the bioavailability of 2,4,6-trinitrotoluene and its primary transformation products in sediment and water. *Environmental Toxicology and Chemistry* 24: 1059-1066.

Crocker, F. H., K. J. Indest, and H. L. Fredrickson. 2006. Biodegradation of the cyclic nitramine explosives RDX, HMX, and CL-20. *Applied Microbiology Biotechnology* 73: 274-290.

Daniels, W. D. 1999. *Soil survey of Perry County, Mississippi.* Natural Resources Conservation Service, Interim Publication. 206 pp.

Day, P. R. 1956. Report of the committee on physical analyses (1954-1955). *Soil Science Society of America Proceedings* 20: 167-169.

Dontsova, K. M., S. L. Yost, J. Simunek, J. C. Pennington, and C. W. Williford. 2006. Dissolution and transport of TNT, RDX, and Composition B in saturated soil columns. *Journal of Environmental Quality* 35: 2043-2054.

Fernando, T., J. A. Bumpus, and S. D. Aust. 1990. Biodegradation of TNT (2,4,6-trinitrotoluene) by *Phanerochaete chrysosporium*.*Applied Environmental Microbiology* 56: 1666-1671.

Frank, K., D. Beegle, and J. Denning. 1998. Phosphorus. In *Recommended Chemical Soil Test Procedures for the North Central Region*, 21-23. NCR Publication No. 221. Columbia, MO: Missouri Agricultural Experiment Station.

Harvey, S. D., R. J. Fellows, D. A. Cataldo, and R. M. Bean. 1991. Fate of the explosive hexahydro-1,3,5-trinitro- 1,3,5-triazine (RDX) in soil and bioaccumulation in bush bean hydroponic plants. *Environmental Toxicology Chemistry* 10: 845-855.

Hawari, J., A. Halasz, E. Paquet, E. Zhou, B. Spencer, G. Ampleman, and S. Thiboutot. 1998. Characterization of metabolites in the biotransformation of 2,4,6-trinitrotoluenewith anaerobic sludge: Role of triaminotoluene. *Applied Environmental Microbiology* 64: 2200-2206.

Hawari, J., A. Halasz, S. B. Beaudet, G. Ampleman, and S. Thiboutot. 1999. Biotransformation of 2,4,6-trinitrotoluene (TNT) with *Phanerochaete chrysosporium* in agitated cultures at pH 4.5. *Applied Environmental Microbiology* 65: 2977-2986.

Hawari, J. 2000. Biodegradation of RDX and HMX: From basic research to field application. In *Biodegradation of Nitroaromatic Compounds and Explosives*, ed. J. C. Spain, J. B. Hughes, and H. J. Knackmuss, Chapter 11: 277-310. Boca Raton, FL: Lewis Publishers.

Hughes, J. B., J. Shanks, M. Vanderford, J. Lauridzen, and R. Bhadra. 1997. Transformation of TNT by aquatic plants and plant tissue cultures. *Environmental Science and Technology* 31: 266-2711.

Jenkins, T. F., P. H. Miyares, K. F. Myers, E.F. McCormick, A. B. Strong. 1995. Comparison of solid phase extraction with salting-out solvent extraction for preconcentration of nitroaromatic and nitramine explosives from water. *Analytica Chimica Acta* 289: 69-78.

Jones, A. M., C. W. Greer, G. Ampleman, S. Thiboutot, J. Lavigne, and J. Hawari. 1995. Biodegradability of selected highly energetic pollutants under aerobic conditions. In *Proceedings of the 3rd International In Situ and On Site Bioreclamation Symposium*, Battelle Press: Columbus, OH: 251-257.

Just, C. L., and J. L. Schnoor. 2004. Phytophotolysis of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in leaves of reed canary grass. *Environmental Science and Technology* 38: 290-295.

Keeney, D. R., and D. W. Nelson. 1982. Nitrogen – inorganic forms. In *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties*. 2nd ed. A.L. Page et al., 643-698. Madison, WI: SSSA Book Series 5, ASA, SSSA.

Larson, S. L., R. P. Jones, L. Escalon, D. Parker. 1999. Classification of explosives transformation products in plant tissue. *Environmental Toxicology and Chemistry* 18: 1270-1276.

Lynch, J. C. 2002. "Dissolution kinetics of high explosive compounds (TNT, RDX, HMX)," ERDC EL TR-02-23. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Mayer, P., W. H. J. Vaes, F. Wijnker, K. C. H. M. Legierse, R. H. Kraaij, J. Tolls, and J. L. M. Hermens. 2000. Sensing dissolved sediment porewater concentrations of persistent and bioaccumulative pollutants using disposable solid-phase microextraction fibers. *Environmental Science and Technology* 34: 5177-5183.

McCutcheon, S. C., and J.L. Schnoor. 2003. *Phytoremediation: Transformation and Control of Contaminants.* John Wiley & Sons.

Monteil-Rivera, F., L. Pauet, S. Deschamps, V. K. Balakrishnan, C. Beaulieu, and J. Hawari. 2004. Physicochemical measurements of CL-20 for environmental applications: Comparison with RDX and HMX. *Journal of Chromatography A*. 1025: 125-132.

Nishino, S. F., J. C. Spain, and H. Zhingqi. 2000. Strategies for aerobic degradation of nitroaromatic compounds by bacteria: process discovery to field application. In *Biodegradation of Nitroaromatic Compounds and Explosives*, ed. J. C. Spain, J. B. Hughes, and H. J. Knackmuss, Chapter 2:7-62. Boca Raton, FL: Lewis Publishers.

Palazzo, A. J., and D. C. Legett. 1986. Effect and disposition of TNT in a terrestrial plant. *Journal of Environmental Quality* 15: 49-52.

Patrick, W. H., Jr. 1958. Modification of method of particle size analysis. *Soil Science Society of America Proceedings* 22: 366-367.

Paterson, S., D. MacKay, and C. McFarlane. 1994. A model of organic chemical uptake by plants from soil and the atmosphere. *Environmental Science and Technology* 28: 2259-2266.

Pennington, J. C., T. F. Jenkins, J. M. Brannon, J. Lynch, T. A. Ranney, T. E. Berry, C. A. Hayes, P. H. Miyares, M. E. Walsh, A. D. Hewitt, N. Perron, and J. J. Delfino. 2001. *Distribution and fate of energetics on DoD test and training ranges: Interim Report 1.* ERDC TR-01-13. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Pinkart, H. C., D. B. Ringelberg, Y. M. Piceno, S. J. MacNaughton, and D. C. White. 2002. Biochemical approaches to biomass measurements and community structure analysis, In *Manual of Environmental Microbiology*, ed. C. J. Hurst, R. L. Crawford, G.R. Kneuden, M.J. McInerney, and L.D. Stetzenbach, 101-113. Washington, DC: American Society of Microbiology Press.

Platzner, I. T., K. Habfast, A. J. Walder, and A. Goetz. 1999. *Modern isotope ratio mass spectrometry*. Chemical Analysis. A Series of Monographs on Analytical Chemistry and its Applications. John Wiley and Sons: 229-235.

Price, C. B., J. M. Brannon, S. L. Yost, and C. A. Hayes. 2000. *Adsorption and transformation of explosives in low-carbon aquifer soils.* ERDC/EL TR-00-11, Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Rieger, P. -G., and H. -J. Knackmuss. 1995. Basic knowledge and perspectives on biodegradation of 2,4,6-trinitrotoluene and related nitroaromatic compounds in contaminated soil. In *Biodegradation of Nitroaromatic Compounds*, ed. J. C. Spain, Chapter 1:1-18. Boca Raton, FL: Lewis Publishers.

Rocheleau, S., R. G. Kuperman, M. Martel, L. Pauet, G. Bardai, S. Wong, M. Sarrazin, S. Dodard, P. Gong, J. Hawari, R. T. Checkai, and G. I. Sunahara. 2006. Phytotoxicity of nitroaromatic compounds freshly amended or weathered and aged in sandy loam soil. *Chemosphere* 62: 545-558.

Sandermann, J. H. 1994. Higher plant metabolism of xenobiotics: the 'green liver' concept. *Pharmacogenetics* 4: 225-241.

Schnoor, J. L., B. Van Aken, L. B. Brentner, S. Tanaka, B. Flokstra, and J. M. Yoon. 2006. Identification of metabolic routes and catabolic enzymes involved in phytoremediation of the nitro-substituted explosives TNT, RDX, and HMX. Final Technical Report. SERDP Project Number 02 CU3-17. July 2006.

Schoenmuth, B. W., and W. Pestemer. 2004. Dendroremediation of trinitrotoluene (TNT). Part 1. Litarature overview and research concept. *Environmental Science and Pollution Research* 11: 273-278.

Schroeder, P., C. Fisher, R. Debus, and A. Wentzel. 2003. Reaction of detoxification mechanisms in suspension ocultured spruce cells (*Picea abies* L.KARST.) to heavy metals in pure mixture and in soil eluates. *Environmental Science and Pollution Research* 10: 225-234.

Sens, C., P. Scheidemann, and D. Werner. 1999. The distribution of ^{14}C-TNT in different biochemical compartments of the monocotyledonous *Triticum aestivum*. *Environmental Pollution* 104: 113-119.

Simini, M., R. S. Wentsel, R. T. Checkai, C. T. Phillips, N. A. Chester, M. A. Major, and J. C. Amos. 1995. Evaluation of soil toxicity at Joliet Army Ammunition Plant. *Environmental Toxicology and Chemistry* 14: 623-630.

Supelco. 1997. SPME/HPLC interface combines fast sample extraction with efficient analysis for explosives (Application Note 98). Bellefonte, PA: Supelco/Sigma Aldrich.

Talmage, S. S., D. M. Opresko, C. J. Maxwell, C. J. E. Welsh, F. M. Cretella, P. H. Reno, and F. B. Daniel. 1999. Nitroaromatic munition compounds: Environmental effects and screening values. *Review in Environmental and Contaminant Toxicology* 161: 1-156.

Thompson, P. L., L. A. Ramer, and J. L. Schnoor. 1998. Uptake and transformation of TNT by hybrid poplar trees. *Environmental Science and Technology* 32: 975-980.

Thompson, P. L., L. A. Ramer, and J. L. Schnoor. 1999. Hexahydro-1,3,5-trinitro- 1,3,5-triazine translocation in poplar trees. *Environmental Toxicology Chemistry* 18: 279-284.

Trapp, S. 2000. Modeling uptake into roots and subsequent translocation of neutral and ionisable organic compounds. *Pesticide Management Science* 56: 767-778.

Trapp, S., C. MacFarlane, and M. Matthies. 1994. Model for uptake of xenobiotics into plants: Validation with bromeacil experiments. *Environmental Toxicology & Chemistry* 13: 413-422.

U.S. Environmental Protection Agency (USEPA). 2006. Nitroaromatics, nitramines, and nitrate esters by high performance liquid chromatography (HPLC) Method 8330B. In *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods.* SW-846. Washington, DC: Office of Solid Waste and Emergency Response. http://www.epa.gov/epawaste/hazard/testmethods/sw846/index.htm

Van Aken, B., J. M. Yoon, C. L. Just, and J. L. Schnoor. 2004. Metabolism and mineralization of hexahydro-1,3,5-trinitro-1,3,5-triazine inside poplar tissues (*Populus deltoides* x *nigra* DN-34). *Environmental Science and Technology* 38: 4572-4579.

Vanderford, M., J. V. Shanks, and J. B. Hughes. 1997. Phytotransformation of trinitrotoluene (TNT) and distribution of metabolic products in *Myriophyllum aquaticum*. *Biotechnology Letters* 19: 277-280.

Verbruggen, E. M. J., W. H. J. Vaes, T. F. Parkerton, and J. L. M. Hermens. 2000. Polyacrylate-coated SPME fibers as a tool to simulate body residues and target concetrations of complex organic mixtures for estimation of baseline toxicity. *Environmental Science and Technology* 34: 324-331.

Walsh, M. E., T. F. Jankins, and P. G. Thorne. 1995. Laboratory and field analytical methods for explosives residues in soil. *Proceedings of the Symposium on Alternatives to Incineration for Disposal of Chemical Munitions and Energetics*, Volume 2: 17.

Warncke, D., and J. R. Brown. 1998. Potassium and other basic cations. In: *Recommended Chemical Soil Test Procedures for the North Central Region.* NCR Publication No. 221:31-33. Columbia, MO: Missouri Agricultural Experiment Station.

Watson, M. E., and J. R. Brown. 1998. pH and lime requirement. In: *Recommended Chemical Soil Test Procedures for the North Central Region.* NCR Publication No. 221:13-16. Columbia, MO: Missouri Agricultural Experiment Station.

Wells, J. B., and R. P. Lanno. 2000. Passive sampling devices (PSDs) as biological surrogates for estimating bioavailability of organic chemical sin soil. In *Environmental Toxicology and Risk Assessment Science, Policy, and Standardization – Implications for Environmental Decisions,* 10th ed.; ASTM STP 1403; ed. B. M. Greenber, R. N. Hull, M. H. Roberts, Jr., and R. W. Genssemer, 253-370. West Conshoshocken, PA: American Society for Testing.

Yoon, J. M., B. Van Aken, and J. L. Schnoor. 2006. Leaching of contaminated leaves following uptake and phytoremediation of RDX, HMX, and TNT by poplar. *International Journal of Phytoremediation* 8: 81-94.

Appendix A: Solid Phase Microextraction Analysis of TNT and RDX

An SPME approach was used to provide a biomimetic, non-specific, assessment of the bioavailabilities of TNT and RDX in this experiment, this in contrast to *S. nutans* roots, which exhibit a specific assessment.

To expose SPMEs to TNT and RDX, SPME disks were immersed in 10-mL volumes of explosives containing water in 15-mL glass vials, capped, and strongly agitated on a shaker (Model Innova 2050, New Brunswick Scientific, NJ) at 168 RPM for 24 hr in darkness at room temperature (approximately 22 ^{o}C). As explosives and their degradation products are photosensitive, all work was conducted under non-UV-emitting fluorescent lighting. In all method verification experiments, SPMEs adsorbed less than 5% (on mg L^{-1} basis) of the compounds present (Mayer et al. 2000; Verbruggen et al. 2000). This minimal sampling prevents the SPME adsorption process from becoming an exhaustive extraction, thus meeting requirements for its use as a 'negligible depletion' biomimetic device. After exposing the fiber, the SPME disk was removed from the water, freed from adhering solution by touching a paper tissue, and placed into an HPLC autosampler vial containing 1.2 mL of 50:50 HPLC-grade acetonitrile:ultrapure water for 90 min to desorb compounds from the fibers. The SPME extracts were analyzed using HPLC as described previously.

Method exploration prior to application

Two experiments were conducted. In the first experiment, an exposure period was determined long enough for the explosives in solution to reach equilibrium with those adsorbed on the 2-cm fiber length inserted into the disk. In the second experiment, a relationship was determined between explosives concentrations in solution and those adsorbed on the 2-cm fiber length inserted into the disk.

Exposure period experiment

Two 10-mg L^{-1} stock solutions of TNT and RDX were prepared by adding 1 mL standard in acetonitrile to 99 mL ultrapure water. From these stock solutions,

1:1 diluted TNT and RDX solutions in water were prepared to create 5-mg L^{-1} concentrations, and 10-mL aliquots were poured in 15-mL glass vials. The SPME disks were immersed in the vials, capped, and agitated on a shaker for periods of 12, 24, 48, and 72 hr. The vials on the shaker were completely covered with aluminum foil during the entire exposure period, except for the times when selected vials were removed. At the end of the exposure periods, the SPME disks were removed from the water, desorbed, and the explosives were analyzed as described above. For one exposure period, i.e., 24 hr, an extra set of vials was used to evaluate desorption in pure acetonitrile. The experiment was replicated three times. Results indicated that explosives concentrations adsorbed on the SPME fibers did not differ significantly (ANOVA; $p>0.05$) with exposure period (Table A1). TNT desorbed from the SPME disks in 50:50 acetonitrile: water exceeded that of RDX by a factor of 2. However, when desorbed in acetonitrile the amounts of TNT and RDX were similar (TNT 2.45 ± 0.08 or 5.0% of initial, RDX 2.68 ± 0.08 or 4.5% of initial). Thus, adsorption of both compounds is similar, but desorption of RDX is a factor of 2 less than that of TNT in 50:50 acetonitrile:water. SPME disks adsorbed less than 5% of the total explosives content of the vials. Based on these results, a 24-hr exposure period was selected to be used for the SPME application in the lysimeter experiment.

Explosives concentration dependence experiment

SPME disks were exposed to solutions of 0.1, 1, 5, and 10 mg L^{-1} TNT and 0.1, 2, 5, and 10 mg L^{-1} RDX for 24 hr. The exposure solutions were prepared by adding the following volumes of a 10-mg L^{-1} stock solution to a final 50-mL solution: 0.5 mL resulting in 0.1 mg L^{-1}, 5 mL resulting in 1 mg L^{-1}, 25 mL resulting in 5 mg L^{-1}, and undiluted for 10 mg L^{-1}. The experiment was replicated three times. Results indicated that desorbable SPME-TNT increased linearly with aqueous TNT concentration between 0.1 and 10 mg TNT L^{-1}, but desorbable SPME-RDX over a smaller trajectory, i.e., between 1 and 5 mg RDX L^{-1}. RDX was not desorbable at RDX concentrations <1 mg L^{-1}, and the desorbable amount of RDX remained the same at RDX concentrations >5 mg L^{-1}. Based on these results, it was concluded that the SPME method detection limits were 0.1 mg mL^{-1} for TNT and 1 mg L^{-1} for RDX, with a linear – but different-response between 0.1 and 10 mg L^{-1} for TNT and between 1 and 5 mg L^{-1} for RDX. Thus, the SPME disks applied by 24 hr exposure to the leachates of the lysimeter experiment and desorbed in 50:50 acetonitrile:water may provide a reasonable indication of TNT and RDX bioavailability for TNT and RDX in the leachates, since the aqueous TNT concentrations were most of the time >0.1 and <10 mg L^{-1} and aqueous RDX >1.0 and <3.5 mg L^{-1} (Figure A1).

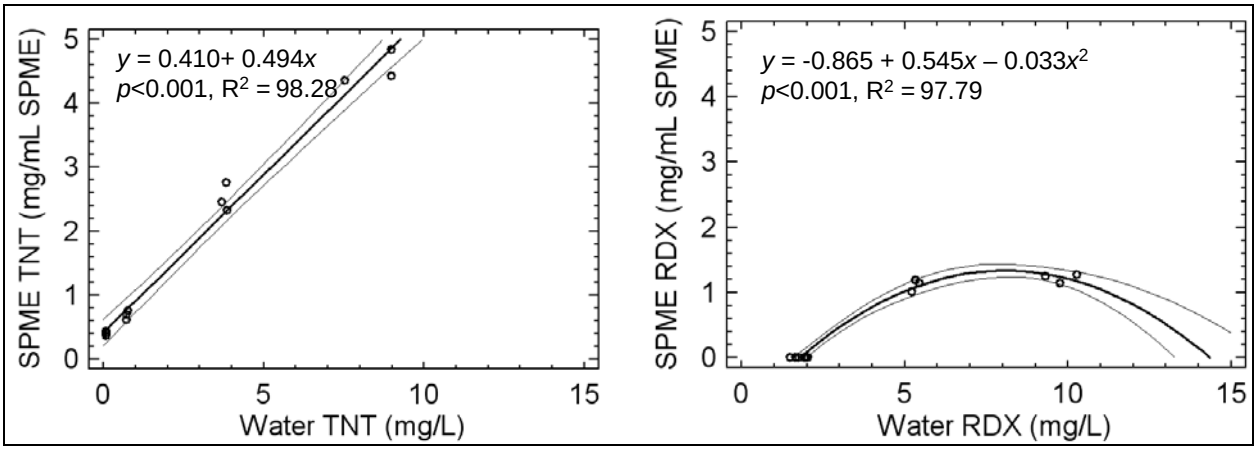

Figure A1. Relationship between explosives concentration in SPME fibers and in water. Regression lines and 95% confidence limits indicated; *y* = SPME response, *x* = target comp-B concentration.

Table A1. SPME TNT and RDX residues over time. Fibers were exposed to an aqueous solution spiked with 5 mg L^{-1} TNT or RDX. Mean values and standard deviations are shown (N=3). ANOVA indicated no significant differences between SPME residues and exposure time (p values of 0.159 for TNT SPMEs and of 0.059 for RDX SPMEs, respectively).

Exposure Time	SPME TNT		SPME RDX	
(h)	(mg mL^{-1}SPME)	(% initial aqueous)	(mg mL^{-1}SPME)	(% initial aqueous)
0	0	0	0	0
12	1.95 ± 0.11	3.96 ± 0.22	1.09 ± 0.06	1.60 ± 0.08
24	2.28 ± 0.12	4.65 ± 0.24	1.18 ± 0.04	1.73 ± 0.06
48	2.09 ± 0.20	4.27 ± 0.40	1.22 ± 0.09	1.79 ± 0.13
72	2.27 ± 0.27	4.62 ± 0.54	1.24 ± 0.03	1.82 ± 0.04

Table A2. Biomass characteristics of *S. nutans* in response to 92 days of exposure to comp-B. Mean values and standard deviations are shown (N=3).

Comp-B Exposure	Block	Shoot Biomass (g dry wt m^{-2})	Root Biomass (g dry wt m^{-2})	Plant Biomass (g dry wt m^{-2})	S:R Ratio	Root Length (m g^{-1} dry wt)	Root Diameter (mm)
	1	349.4	42.0	391.4	8.3	2.7	0.54 ± 0.05
	2	99.0	37.1	136.1	2.7	3.2	0.74 ± 0.08
	3	197.3	52.0	249.3	3.8	3.5	0.66 ± 0.37
Mean ± SD		215.2 ± 126.2	43.7 ± 7.6	258.9 ± 127.9	4.9 ± 3.0	3.2 ± 0 .4	0.65 ± 0.10
73 mg kg^{-1}	1	166.5	45.9	212.4	3.6	1.6 ± 0.4	0.76 ± 0.15
73 mg kg^{-1}	2	233.2	34.9	268.1	6.7	5.9 ± 4.2	0.56 ± 0.05
73 mg kg^{-1}	3	306.1	88.0	394.1	3.5	2.1 ± 0.6	0.66 ± 0.12
Mean ± SD		235.3 ± 69.8	56.3 ± 28.0	291.5 ± 93.1	4.6 ± 1.8	3.2 ± 2.4	0.66 ± 0.10
146 mg kg^{-1}	1	199.1	48.9	247.9	4.1	2.1 ± 0	0.99 ± 0

Comp-B Exposure	Block	Shoot Biomass (g dry wt m^{-2})	Root Biomass (g dry wt m^{-2})	Plant Biomass (g dry wt m^{-2})	S:R Ratio	Root Length (m g^{-1} dry wt)	Root Diameter (mm)
146 mg kg^{-1}	2	118.5	20.6	139.1	5.8	1.9 ± 1.2	0.52 ± 0.07
146 mg kg^{-1}	3	192.6	71.2	263.8	2.7	2.4 ± 1.0	0.60 ± 0.08
Mean ± SD		170.1 ± 44.8	46.9 ± 25.4	255.9 ± 11.2	4.2 ± 1.5	2.1 ± 0.2	0.70 ± 0.25
218 mg kg^{-1}	1	110.6	10.0	120.6	11.1	3.2 ± 2.6	0.58 ± 0.15
218 mg kg^{-1}	2	79.3	65.1	144.5	1.2	1.8 ± 0.4	0.53 ± 0.11
218 mg kg^{-1}	3	144.5	47.1	191.6	3.1	2.8 ± 2.5	0.65 ± 0.15
Mean ± SD		111.5 ± 32.6	40.7 ± 28.1	152.2 ± 36.1	5.1 ± 5.2	2.6 ± 0.7	0.59 ± 0.06

Table A3. Extractable TNT, 2ADNT, 4ADNT, RDX and MNX concentrations in shoots and roots of *S. nutans* in response to various periods of exposure to comp-B. Mean values and standard deviations are shown (N=3).

Factor	Extractable Explosives Parent and Degradation Compounds in Plants				
Comp-B Exposure, days	TNT (mg kg^{-1} dry wt)	2ADNT (mg kg^{-1} dry wt)	4ADNT (mg kg^{-1} dry wt)	RDX (mg kg^{-1} dry wt)	MNX (mg kg^{-1} dry wt)
Shoots					
73 mg kg^{-1}, 28 d	29.5 ± 8.5	30.1 ± 15.5	15.0 ± 10.5	1143.4 ± 280.3	0.0± 0.0
73 mg kg^{-1}, 63 d	12.2 ± 15.6	60.9 ± 80.0	65.1 ± 51.9	1953.4 ± 374.9	0.0 ± 0.0
73 mg kg^{-1}, 92 d	0.0 ± 0.0	78.5 ± 84.3	23.4 ± 40.5	3981.9 ± 832.9	47.1± 81.6
146 mg kg^{-1}, 28 d	135.3 ± 27.4	76.5 ± 44.6	54.0 ± 37.5	1549.9 ± 215.1	0.0 ± 0.0
146 mg kg^{-1}, 63 d	127.7 ± 85.6	131.0 ± 104.0	106.1± 83.4	1747.0 ± 635.8	44.9 ± 42.0
146 mg kg^{-1}, 92 d	291.6 ± 318.0	502.5 ± 420.7	441.4 ± 384.2	4089.2 ± 283.1	47.2 ± 42.9
218 mg kg^{-1}, 28 d	287.2 ± 25.6	128.5 ± 103.2	95.0 ± 82.3	1606.7 ± 708.1	0.0 ± 0.0
218 mg kg^{-1}, 63 d	357.9 ± 290.5	255.2 ± 213.7	93.2 ± 135.9	1732.8 ± 495.7	45.2 ± 78.2
218 mg kg^{-1}, 92 d	455.2 ± 329.8	774.1 ± 689.6	608.9 ± 541.0	4835.1 ± 2434.1	70.7 ± 61.7
Roots					
73 mg kg^{-1}, 28 d	300.5 ± 98.7	194.5 ± 106.9	170.0 ± 114.8	199.3± 8.4	0.0 ± 0.0
73 mg kg^{-1}, 63 d	120.9 ± 85.9	200.2 ± 200.8	167.1 ± 243.0	1343.7 ± 830.7	0.0 ± 0.0
73 mg kg^{-1}, 92 d	220.0 ± 381.1	165.5 ± 68.9	100.6 ± 96.7	3461.6 ± 1495.7	8.5 ± 14.7
146 mg kg^{-1}, 28 d	1081.0 ± 279.7	308.6 ± 40.5	285.9 ± 43.9	1583.7 ± 460.3	0.0 ± 0.0
146 mg kg^{-1}, 63 d	507.7 ± 162.7	387.3 ± 93.9	237.9 ± 195.3	1854.2 ± 296.0	0.0 ± 0.0
146 mg kg^{-1}, 92 d	667.4 ± 498.9	791.4 ± 504.8	840.7 ± 585.3	3314.2 ± 2640.7	10.0 ± 17.3
218 mg kg^{-1}, 28 d	1290.0 ± 191.2	657.3 ± 614.9	382.1 ± 194.1	1778.2 ± 570.0	0.0 ± 0.0
218 mg kg^{-1}, 63 d	1337.1 ± 69.8	691.4 ± 389.3	303.6 ± 243.0	272.1 ± 1291.7	40.6 ± 70.3
218 mg kg^{-1}, 92 d	2566.9 ± 1709.6	1444.1 ± 1053.7	1420.3 ± 1114.4	5754.2 ± 3607.3	41.9 ± 72.6

Table A4. Extractable TNT, 2ADNT, 4ADNT, RDX and MNX contents (in mg core^{-1}) in shoots, roots, and plants of *S. nutans* in response to 92 days of exposure to comp-B. Mean values and standard deviations are shown (N=3). Listed values multiplied by a factor of 10.

Comp-B Exposure	Block	TNT	2ADNT	4ADNT	RDX	MNX
Shoots						
73 mg kg^{-1}	1	0.0 ± 0.0	32.1 ± 8.7	12.8 ± 5.5	901.5 ± 47.4	25.8 ± 4.3
73 mg kg^{-1}	2	0.0 ± 0.0	9.4 ± 8.8	0.0 ± 0.0	869.4 ± 233.3	0.0 ± 0.0
73 mg kg^{-1}	3	0.0 ± 0.0	7.8 ± 1.3	0.0 ± 0.0	1212.9 ± 198.6	0.0 ± 0.0
Mean ± SD		0.0 ± 0.0	16.4 ± 13.5	4.3 ± 7.4	994.6 ± 189.7	8.6 ± 14.9
146 mg kg^{-1}	1	53.1 ± 2.1	119.4 ± 0.7	110.5 ± 0.8	859.2 ± 53.8	6.7 ± 11.7
146 mg kg^{-1}	2	82.0 ± 0.4	116.9 ± 3.1	102.6 ± 1.8	574.1 ± 24.7	12.5 ± 2.1
146 mg kg^{-1}	3	0.1 ± 0.2	13.0 ± 1.1	6.1 ± 0.7	827.8 ± 37.3	3.1 ± 5.4
Mean ± SD		45.1 ± 41.5	83.1 ± 60.7	73.1 ± 58.1	753.7 ± 156.3	7.4 ± 4.7
218 mg kg^{-1}	1	84.6 ± 0.7	178.9 ± 0.6	138.2 ± 5.2	927.1 ± 52.8	13.8 ± 1.2
218 mg kg^{-1}	2	51.2 ± 2.3	65.2 ± 5.2	55.0 ± 5.2	310.8 ± 50.4	8.5 ± 0.3
218 mg kg^{-1}	3	12.6 ± 1.0	15.1 ± 0.2	8.9 ± 0.4	521.8 ± 27.5	0.0 ± 0.0
Mean ± SD		49.5 ± 36.0	86.5 ± 83.9	67.3 ± 65.4	586.6 ± 313.2	7.5 ± 7.0
Roots						
73 mg kg^{-1}	1	0.0 ± 0.0	12.1 ± 0.8	10.5 ± 0.2	199.3 ± 8.4	1.3 ± 2.2
73 mg kg^{-1}	2	25.3 ± 6.8	5.7 ± 1.0	0.8 ± 0.2	177.9 ± 47.8	0.0 ± 0.0
73 mg kg^{-1}	3	0.0 ± 0.0	10.2 ± 1.5	7.1± 2.9	171.9 ± 28.1	0.0 ± 0.0
Mean ± SD		8.4 ± 14.6	9.4 ± 3.3	6.1 ± 4.9	183.0 ± 14.4	0.4 ± 0.7
146 mg kg^{-1}	1	50.6 ± 2.3	64.5 ± 2.3	69.3 ± 3.6	341.1 ± 17.7	1.6 ± 2.8
146 mg kg^{-1}	2	21.9 ± 2.8	21.3 ± 1.1	23.7 ± 1.8	43.8 ± 1.0	0.0 ± 0
146 mg kg^{-1}	3	7.1 ± 0.9	17.8 ± 1.0	14.0 ± 2.0	128.4 ± 16.2	0.0 ± 0
Mean ± SD		26.5 ± 22.1	34.6 ± 26.0	35.7 ± 29.5	171.1 ± 153.2	0.5 ± 0.9
218 mg kg^{-1}	1	45.9 ± 0.0	27.5 ± 0.0	27.8 ± 0.0	91.6 ± 0.0	0.0 ± 0.0
218 mg kg^{-1}	2	195.6 ± 14.7	102.3 ± 5.5	101.9 ± 5.2	520.1± 12.5	9.0 ± 0.7
218 mg kg^{-1}	3	40.2 ± 7.2	20.5 ± 2.9	15.6 ± 2.2	84.4 ± 12.6	0.0 ± 0.0
Mean ± SD		93.9 ± 88.1	50.1 ± 45.3	48.4 ± 46.7	232.0 ± 249.5	3.0 ± 5.2
Plants						
73 mg kg^{-1}	1	0.0 ± 0.0	44.2± 8.2	23.3 ± 5.5	1100.8 ± 51.2	27.1± 4.0
73 mg kg^{-1}	2	25.3 ± 6.8	15.1 ± 9.8	0.8 ± 0.2	1047.2 ± 281.1	0.0 ± 0.0
73 mg kg^{-1}	3	0.0 ± 0.0	18.1± 2.8	7.1± 2.9	1384.8 ± 226.7	0.0 ± 0.0
Mean ± SD		8.4 ± 14.6	25.7 ± 16.0	10.4 ± 11.6	1177.6 ± 181.4	9.0 ± 15.6
146 mg kg^{-1}	1	103.7± 0.6	183.9 ± 1.6	179.8 ± 2.8	1200.3 ± 57.6	8.3 ± 14.5

Comp-B Exposure	Block	TNT	2ADNT	4ADNT	RDX	MNX
Plants						
146 mg kg^{-1}	2	103.9 ± 3.2	183.2 ± 4.0	126.3 ± 3.4	617.9 ± 25.5	12.5 ± 2.1
146 mg kg^{-1}	3	7.3 ± 0.8	30.8 ± 0.2	20.2 ± 1.6	956.3 ± 52.4	3.1± 5.4
Mean ± SD		71.6 ± 55.7	117.6 ± 78.6	108.7 ± 81.3	924.8 ± 292.5	7.9 ± 4.6
218 mg kg^{-1}	1	130.5 ± 0.7	206.3 ± 0.6	165.8 ± 5.2	1018.6 ± 52.8	13.8 ± 1.2
218 mg kg^{-1}	2	246.7± 17.0	167.7± 10.1	157.0 ± 9.2	830.8 ± 52.1	17.5 ± 0.8
218 mg kg^{-1}	3	52.8 ± 7.9	35.6 ±2.8	24.6 ± 2.2	606.3 ± 22.1	0.0 ± 0.0
Mean ± SD		143.3 ± 97.5	136.5 ± 89.5	115.7 ± 79.1	818.5 ± 206.4	10.4 ± 9.2

Table A5. Extractable TNT, 2ADNT, and 4ADNT concentrations in soil cores after 92 days of exposure to comp-B. Measured values, means and standard deviations are shown (N=3).

Comp-B Exposure	Initial TNT (mg kg^{-1} dry wt)	Block	Layer	Final TNT (mg kg^{-1}dry wt)	Final 2ADNT (mg kg^{-1}dry wt)	Final 4ADNT (mg kg^{-1}dry wt)
Bare						
73 mg kg^{-1}	23.4	1	Top	5.9	0.091	0.0
73 mg kg^{-1}	23.4	1	Mid	2.4	0.0	0.0
73 mg kg^{-1}	23.4	1	Bottom	0.5	0.0	0.0
73 mg kg^{-1}	23.4	2	Top	8.4	1.0	0.0
73 mg kg^{-1}	23.4	2	Mid	0.9	0.0	0.0
73 mg kg^{-1}	23.4	2	Bottom	5.0	0.0	0.0
73 mg kg^{-1}	23.4	3	Top	4.4	1.5	0.8
73 mg kg^{-1}	23.4	3	Mid	0.4	0.0	0.0
73 mg kg^{-1}	23.4	3	Bottom	0.1	0.0	0.0
Mean-L ± SD	23.4 ± 7.1		Top	6.2 ± 2.0	0.9 ± 0.7	0.3 ± 0.5
Mean-L ± SD	23.4 ± 7.1		Mid	1.2 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
Mean-L ± SD	23.4 ± 7.1		Bottom	1.9 ± 2.7	0.0 ± 0.0	0.0 ± 0.0
146 mg kg^{-1}	47.9	1	Top	31.3	1.2	0.4
146 mg kg^{-1}	47.9	1	Mid	0.5	0.0	0.0
146 mg kg^{-1}	47.9	1	Bottom	1.7	0.0	0.0
146 mg kg^{-1}	47.9	2	Top	35.6	1.4	0.4
146 mg kg^{-1}	47.9	2	Mid	0.5	0.0	0.0
146 mg kg^{-1}	47.9	2	Bottom	0.0	0.0	0.0
146 mg kg^{-1}	47.9	3	Top	91.6	2.6	1.5

Comp-B Exposure	Initial TNT (mg kg^{-1} dry wt)	Block	Layer	Final TNT (mg kg^{-1}dry wt)	Final 2ADNT (mg kg^{-1}dry wt)	Final 4ADNT (mg kg^{-1}dry wt)
Bare						
146 mg kg^{-1}	47.9	3	Mid	15.4	0.6	0.0
146 mg kg^{-1}	47.9	3	Bottom	2.2	0.0	0.0
Mean-L ± SD	47.9 ± 7.1		Top	52.9 ± 33.6	1.8 ± 0.8	0.8 ± 0.6
Mean-L ± SD	47.9 ± 7.1		Mid	5.5 ± 8.6	0.2 ± 0.3	0.0 ± 0.0
Mean-L ± SD	47.9 ± 7.1		Bottom	1.3 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
218 mg kg^{-1}	65.5	1	Top	33.8	1.0	0.138
218 mg kg^{-1}	65.5	1	Mid	16.1	0.5	0.0
218 mg kg^{-1}	65.5	1	Bottom	24.6	0.5	0.0
218 mg kg^{-1}	65.5	2	Top	110.4	1.4	0.6
218 mg kg^{-1}	65.5	2	Mid	4.8	0.4	0.0
218 mg kg^{-1}	65.5	2	Bottom	4.2	1.8	0.1
218 mg kg^{-1}	65.5	3	Top	76.2	1.4	0.4
218 mg kg^{-1}	65.5	3	Mid	77.6	1.0	0.0
218 mg kg^{-1}	65.5	3	Bottom	8.0	0.0	0.0
Mean-L ± SD	65.5 ± 5.2		Top	73.5 ± 38.4	1.3 ± 0.3	0.4 ± 0.2
Mean-L ± SD	65.5 ± 5.2		Mid	32.9 ± 39.2	0.6 ± 0.3	0.0 ± 0.0
Mean-L ± SD	65.5 ± 5.2		Bottom	12.3 ± 10.8	0.2 ± 0.2	0.0 ± 0.0
***S. nutans* Vegetated**						
73 mg kg^{-1}	23.4	1	Top	0.0	1.1	0.0
73 mg kg^{-1}	23.4	1	Mid	0.0	0.0	0.0
73 mg kg^{-1}	23.4	1	Bottom	0.0	0.0	0.0
73 mg kg^{-1}	23.4	2	Top	9.7	2.0	1.0
73 mg kg^{-1}	23.4	2	Mid	0.1	0.0	0.0
73 mg kg^{-1}	23.4	2	Bottom	0.0	0.0	0.0
73 mg kg^{-1}	23.4	3	Top	2.2	3.1	2.1
73 mg kg^{-1}	23.4	3	Mid	0.1	0.0	0.0
73 mg kg^{-1}	23.4	3	Bottom	0.4	0.0	0.0
Mean-L ± SD	23.4 ± 7.1		Top	4.0 ± 5.1	2.1 ± 1.0	1.1 ± 1.0
Mean-L ± SD	23.4 ± 7.1		Mid	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Mean-L ± SD	23.4 ± 7.1		Bottom	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0

Comp-B Exposure	Initial TNT (mg kg^{-1} dry wt)	Block	Layer	Final TNT (mg kg^{-1}dry wt)	Final 2ADNT (mg kg^{-1}dry wt)	Final 4ADNT (mg kg^{-1}dry wt)
S. nutans Vegetated						
146 mg kg^{-1}	47.9	1	Top	3.2	1.7	0.8
146 mg kg^{-1}	47.9	1	Mid	0.2	0.0	0.0
146 mg kg^{-1}	47.9	1	Bottom	4.6	0.0	0.0
146 mg kg^{-1}	47.9	2	Top	10.7	0.4	0.3
146 mg kg^{-1}	47.9	2	Mid	3.7	0.7	0.0
146 mg kg^{-1}	47.9	2	Bottom	0.0	0.0	0.0
146 mg kg^{-1}	47.9	3	Top	50.9	1.1	0.5
146 mg kg^{-1}	47.9	3	Mid	28.6	0.6	0.0
146 mg kg^{-1}	47.9	3	Bottom	3.3	0.1	0.0
Mean-L ± SD	47.9 ± 7.1		Top	21.6 ± 25.6	1.1 ± 0.6	0.5 ± 0.3
Mean-L ± SD	47.9 ± 7.1		Mid	10.9 ± 15.5	0.5 ± 0.4	0.0 ± 0.0
Mean-L ± SD	47.9 ± 7.1		Bottom	2.6 ± 2.4	0.0 ± 0.0	0.0 ± 0.0
218 mg kg^{-1}	65.5	1	Top	77.1	0.7	0.5
218 mg kg^{-1}	65.5	1	Mid	6.4	0.3	0.1
218 mg kg^{-1}	65.5	1	Bottom	8.4	0.6	0.0
218 mg kg^{-1}	65.5	2	Top	90.1	2.1	0.7
218 mg kg^{-1}	65.5	2	Mid	49.3	0.8	0.0
218 mg kg^{-1}	65.5	2	Bottom	6.4	0.0	0.0
218 mg kg^{-1}	65.5	3	Top	101.1	3.1	2.0
218 mg kg^{-1}	65.5	3	Mid	7.6	1.1	0.1
218 mg kg^{-1}	65.5	3	Bottom	6.0	0.8	0.0
Mean-L ± SD	65.5 ± 5.2		Top	89.4 ± 12.0	2.0 ± 1.2	1.0 ± 0.8
Mean-L ± SD	65.5 ± 5.2		Mid	21.1 ± 24.4	0.7 ± 0.4	0.1 ± 0.1
Mean-L ± SD	65.5 ± 5.2		Bottom	6.9 ± 1.3	0.5 ± 0.4	0.0 ± 0.0

Table A6. Extractable TNT, 2ADNT and 4ADNT contents in soil cores after 92 days of exposure to comp-B. Measured values, means and standard deviations are shown (N=3).

Comp-B Exposure	Initial TNT (mg core^{-1})	Block	Layer	Final TNT (mg core^{-1})	Final 2ADNT (mg core^{-1})	Final 4ADNT (mg core^{-1})
Bare						
73 mg kg^{-1}		1	Top	72.9	1.1	0.0
73 mg kg^{-1}		1	Mid	29.4	0.0	0.0
73 mg kg^{-1}		1	Bottom	26.5	0.0	0.0
73 mg kg^{-1}	1770	1	Total	128.9	1.1	0.0
73 mg kg^{-1}		2	Top	105.0	13.0	0.0
73 mg kg^{-1}		2	Mid	10.7	0.0	0.0
73 mg kg^{-1}		2	Bottom	255.8	0.0	0.0
73 mg kg^{-1}	1770	2	Total	371.6	13.0	0.0
73 mg kg^{-1}		3	Top	55.4	18.7	9.8
73 mg kg^{-1}		3	Mid	4.4	0.0	0.0
73 mg kg^{-1}		3	Bottom	3.4	0.0	0.0
73 mg kg^{-1}	1770	3	Total	63.1	18.7	9.8
Mean-L ± SD	1770± 527		Top	77.7 ± 25.2	11.0 ± 9.0	3.3 ± 5.6
Mean-L± SD	1770± 527		Mid	14.9 ± 13.0	0.0 ± 0.0	0.0 ± 0.0
Mean-L± SD	1770± 527		Bottom	95.3 ± 139.5	0.0 ± 0.0	0.0 ± 0.0
Mean-T± SD	1770± 527		Total	187.9 ± 162.5	11.0 ± 9.0	3.3 ± 5.6
146 mg kg^{-1}		1	Top	389.8	15.2	4.4
146 mg kg^{-1}		1	Mid	6.5	0.0	0.0
146 mg kg^{-1}		1	Bottom	84.5	0.0	0.0
146 mg kg^{-1}	3633	1	Total	480.8	15.2	4.4
146 mg kg^{-1}		2	Top	443.8	17.9	5.6
146 mg kg^{-1}		2	Mid	6.2	0.0	0.0
146 mg kg^{-1}		2	Bottom	1.9	0.0	0.0
146 mg kg^{-1}	3633	2	Total	452.0	17.9	5.6
146 mg kg^{-1}		3	Top	1140.5	33.0	18.7
146 mg kg^{-1}		3	Mid	191.4	7.5	0.0
146 mg kg^{-1}		3	Bottom	112.7	0.0	0.0
146 mg kg^{-1}	3633	3	Total	1444.6	40.5	18.7
Mean-L ± SD	3633 ± 539		Top	658.1 ± 418.7	22.0 ± 9.6	9.6 ± 7.9
Mean-L± SD	3633 ± 539		Mid	68.0 ± 106.8	2.5 ± 4.4	0.0 ± 0.0

Comp-B Exposure	Initial TNT (mg core^{-1})	Block	Layer	Final TNT (mg core^{-1})	Final 2ADNT (mg core^{-1})	Final 4ADNT (mg core^{-1})
Bare						
Mean-L± SD	3633 ± 539		Bottom	66.4 ± 57.5	0.0 ± 0.0	0.0 ± 0.0
Mean-T± SD	3633 ± 539		Total	792.5 ± 565.0	24.5 ± 13.9	9.6 ± 7.9
218 mg kg^{-1}		1	Top	420.7	11.8	1.7
218 mg kg^{-1}		1	Mid	200.6	6.1	0.0
218 mg kg^{-1}		1	Bottom	1250.5	24.0	0.0
218 mg kg^{-1}	4964	1	Total	1871.7	41.9	1.7
218 mg kg^{-1}		2	Top	1374.9	17.2	7.1
218 mg kg^{-1}		2	Mid	60.0	5.1	0.0
218 mg kg^{-1}		2	Bottom	214.1	9.0	3.5
218 mg kg^{-1}	4964	2	Total	1649.1	31.4	10.6
218 mg kg^{-1}		3	Top	948.2	17.7	5.1
218 mg kg^{-1}		3	Mid	966.4	12.8	0.0
218 mg kg^{-1}		3	Bottom	406.2	0.0	0.0
218 mg kg^{-1}	4964	3	Total	2320.8	30.5	5.1
Mean-L ± SD	4964± 397		Top	914.6 ± 478.0	15.6 ± 3.3	4.6 ± 2.7
Mean-L± SD	4964± 397		Mid	409.0 ± 487.8	8.0 ± 4.1	0.0 ± 0.0
Mean-L± SD	4964± 397		Bottom	623.6 ± 551.3	11.0 ± 12.1	1.2 ± 2.0
Mean-T± SD	4964± 397		Total	1947.2 ± 342.1	34.6 ± 6.4	5.8 ± 4.5
***S. nutans* Vegetated**						
73 mg kg^{-1}		1	Top	0.0	13.2	0.3
73 mg kg^{-1}		1	Mid	0.0	0.0	0.0
73 mg kg^{-1}		1	Bottom	0.0	0.0	0.0
73 mg kg^{-1}	1770	1	Total	0.0	13.2	0.3
73 mg kg^{-1}		2	Top	121.0	25.2	13.1
73 mg kg^{-1}		2	Mid	0.8	0.0	0.0
73 mg kg^{-1}		2	Bottom	1.0	0.0	0.0
73 mg kg^{-1}	1770	2	Total	122.7	25.2	13.1
73 mg kg^{-1}		3	Top	27.0	38.4	25.9
73 mg kg^{-1}		3	Mid	1.7	0.0	0.0
73 mg kg^{-1}		3	Bottom	21.5	0.0	0.0
73 mg kg^{-1}	1770	3	Total	50.2	38.4	25.9

Comp-B Exposure	Initial TNT (mg core^{-1})	Block	Layer	Final TNT (mg core^{-1})	Final 2ADNT (mg core^{-1})	Final 4ADNT (mg core^{-1})
S. nutans Vegetated						
Mean-L ± SD	1770± 527		Top	49.3 ± 63.5	25.6 ± 12.6	13.1 ± 12.8
Mean-L± SD	1770± 527		Mid	0.8 ± 0.9	0.0 ± 0.0	0.0 ± 0.0
Mean-L± SD	1770± 527		Bottom	7.5 ± 12.1	0.0 ± 0.0	0.0 ± 0.0
Mean-T± SD	1770± 527		Total	57.6 ± 61.7	25.6 ± 12.6	13.1 ± 12.8
146 mg kg^{-1}		1	Top	40.0	21.3	10.3
146 mg kg^{-1}		1	Mid	3.0	0.0	0.0
146 mg kg^{-1}		1	Bottom	232.4	0.0	0.0
146 mg kg^{-1}	3633	1	Total	275.4	21.3	10.3
146 mg kg^{-1}		2	Top	132.7	5.2	4.2
146 mg kg^{-1}		2	Mid	46.2	9.2	0.0
146 mg kg^{-1}		2	Bottom	0.0	0.0	0.0
146 mg kg^{-1}	3633	2	Total	178.9	14.4	4.2
146 mg kg^{-1}		3	Top	633.4	13.7	5.9
146 mg kg^{-1}		3	Mid	356.5	7.9	0.0
146 mg kg^{-1}		3	Bottom	169.6	3.1	0.0
146 mg kg^{-1}	3633	3	Total	1159.6	24.7	5.9
Mean-L ± SD	3633 ± 539		Top	268.7 ± 319.2	13.4 ± 8.0	6.8 ± 3.2
Mean-L ± SD	3633 ± 539		Mid	135.3 ± 192.8	5.7 ± 5.0	0.0 ± 0.0
Mean-L ± SD	3633 ± 539		Bottom	134.0 ± 120.2	1.0 ± 1.8	0.0 ± 0.0
Mean-T ± SD	3633 ± 539		Total	538.0 ± 540.5	20.1 ± 5.2	6.8 ± 3.2
218 mg kg^{-1}		1	Top	960.2	8.1	5.7
218 mg kg^{-1}		1	Mid	80.0	3.2	1.2
218 mg kg^{-1}		1	Bottom	429.0	28.5	0.0
218 mg kg^{-1}	4964	1	Total	1469.2	39.8	6.9
218 mg kg^{-1}		2	Top	1122.2	26.7	8.8
218 mg kg^{-1}		2	Mid	613.9	9.6	0.0
218 mg kg^{-1}		2	Bottom	327.7	0.0	0.0
218 mg kg^{-1}	4964	2	Total	2063.9	36.3	8.8
218 mg kg^{-1}		3	Top	1258.1	38.2	24.6
218 mg kg^{-1}		3	Mid	94.1	13.1	1.7

Comp-B Exposure	Initial TNT (mg core^{-1})	Block	Layer	Final TNT (mg core^{-1})	Final 2ADNT (mg core^{-1})	Final 4ADNT (mg core^{-1})
S. nutans Vegetated						
218 mg kg^{-1}		3	Bottom	304.0	40.7	0.0
218 mg kg^{-1}	4964	3	Total	1656.2	91.9	26.3
Mean-L ± SD	4964± 397		Top	113.5 ± 149.2	24.3 ± 15.1	13.0 ± 10.2
Mean-L ± SD	4964± 397		Mid	262.7 ± 304.3	8.6 ± 5.0	1.0 ± 0.9
Mean-L ± SD	4964± 397		Bottom	353.5 ± 66.4	23.1 ± 20.9	0.0 ± 0.0
Mean-T ± SD	4964± 397		Total	1729.7 ± 304.1	56.0 ± 31.2	14.0 ± 10.7

Table A7. Extractable RDX and MNX concentrations in soil cores after 92 days of exposure to comp-B. Measured values, means and standard deviations are shown (N=3).

Comp-B Exposure	Initial RDX (mg kg^{-1} dry wt)	Block	Layer	Final RDX (mg kg^{-1}dry wt)	Final MNX (mg kg^{-1}dry wt)
Bare					
73 mg kg^{-1}	30.3	1	Top	13.5	0.0
73 mg kg^{-1}	30.3	1	Mid	31.6	0.0
73 mg kg^{-1}	30.3	1	Bottom	5.7	0.0
73 mg kg^{-1}	30.3	2	Top	38.3	0.0
73 mg kg^{-1}	30.3	2	Mid	10.0	0.0
73 mg kg^{-1}	30.3	2	Bottom	15.5	0.0
73 mg kg^{-1}	30.3	3	Top	24.2	0.0
73 mg kg^{-1}	30.3	3	Mid	28.1	0.0
73 mg kg^{-1}	30.3	3	Bottom	16.3	0.0
Mean-L ± SD	30.3 ± 4.5		Top	25.3 ± 12.4	0.0 ± 0.0
Mean-L ± SD	30.3 ± 4.5		Mid	23.2 ± 11.6	0.0 ± 0.0
Mean-L ± SD	30.3 ± 4.5		Bottom	12.5 ± 5.9	0.0 ± 0.0
146 mg kg^{-1}	65.2	1	Top	39.6	0.0
146 mg kg^{-1}	65.2	1	Mid	6.4	0.0
146 mg kg^{-1}	65.2	1	Bottom	4.7	0.0
146 mg kg^{-1}	65.2	2	Top	108.7	0.0
146 mg kg^{-1}	65.2	2	Mid	15.8	0.0
146 mg kg^{-1}	65.2	2	Bottom	7.6	0.0
146 mg kg^{-1}	65.2	3	Top	146.8	0.0

Comp-B Exposure	Initial RDX (mg kg^{-1} dry wt)	Block	Layer	Final RDX (mg kg^{-1}dry wt)	Final MNX (mg kg^{-1}dry wt)
Bare					
146 mg kg^{-1}	65.2	3	Mid	67.6	0.0
146 mg kg^{-1}	65.2	3	Bottom	18.5	0.0
Mean-L ± SD	65.2 ± 15.7		Top	98.4 ± 54.3	0.0 ± 0.0
Mean-L ± SD	65.2. ± 15.7		Mid	29.9 ± 32.9	0.0 ± 0.0
Mean-L ± SD	65.2 ± 15.7		Bottom	10.3 ± 7.3	0.0 ± 0.0
218 mg kg^{-1}	85.0	1	Top	69.8	0.0
218 mg kg^{-1}	85.0	1	Mid	42.2	0.0
218 mg kg^{-1}	85.0	1	Bottom	38.5	0.0
218 mg kg^{-1}	85.0	2	Top	216.3	0.7
218 mg kg^{-1}	85.0	2	Mid	29.1	0.0
218 mg kg^{-1}	85.0	2	Bottom	11.8	0.0
218 mg kg^{-1}	85.0	3	Top	167.9	0.0
218 mg kg^{-1}	85.0	3	Mid	189.1	0.9
218 mg kg^{-1}	85.0	3	Bottom	26.7	0.0
Mean-L ± SD	85.0 ± 12.0		Top	151.3 ± 74.7	0.2 ± 0.4
Mean-L ± SD	85.0 ± 12.0		Mid	86.8 ± 86.8	0.3 ± 0.5
Mean-L ± SD	85.0 ± 12.0		Bottom	25.6 ± 13.4	0.0 ± 0.0
S. nutans Vegetated					
73 mg kg^{-1}	30.3	1	Top	69.2	0.0
73 mg kg^{-1}	30.3	1	Mid	4.7	0.0
73 mg kg^{-1}	30.3	1	Bottom	3.9	0.0
73 mg kg^{-1}	30.3	2	Top	78.4	0.0
73 mg kg^{-1}	30.3	2	Mid	3.3	0.0
73 mg kg^{-1}	30.3	2	Bottom	1.4	0.0
73 mg kg^{-1}	30.3	3	Top	56.9	1.4
73 mg kg^{-1}	30.3	3	Mid	4.9	0.1
73 mg kg^{-1}	30.3	3	Bottom	3.6	0.2
Mean-L ± SD	30.3 ± 4.5		Top	68.2 ± 10.8	0.5 ± 0.8
Mean-L ± SD	30.3 ± 4.5		Mid	4.3 ± 0.9	0.0 ± 0.1
Mean-L ± SD	30.3 ± 4.5		Bottom	3.0 ± 1.4	0.1 ± 0.1

Comp-B Exposure	Initial RDX (mg kg^{-1} dry wt)	Block	Layer	Final RDX (mg kg^{-1}dry wt)	Final MNX (mg kg^{-1}dry wt)
S. nutans Vegetated					
146 mg kg^{-1}	65.2	1	Top	52.3	0.0
146 mg kg^{-1}	65.2	1	Mid	10.5	0.0
146 mg kg^{-1}	65.2	1	Bottom	16.6	0.0
146 mg kg^{-1}	65.2	2	Top	64.2	0.1
146 mg kg^{-1}	65.2	2	Mid	87.1	0.0
146 mg kg^{-1}	65.2	2	Bottom	9.7	0.0
146 mg kg^{-1}	65.2	3	Top	159.1	0.0
146 mg kg^{-1}	65.2	3	Mid	99.1	0.0
146 mg kg^{-1}	65.2	3	Bottom	11.3	0.0
Mean-L ± SD	65.2 ± 15.7		Top	91.9 ± 58.5	0.0 ± 0.1
Mean-L ± SD	65.2. ± 15.7		Mid	65.5 ± 48.1	0.0 ± 0.0
Mean-L ± SD	65.2 ± 15.7		Bottom	12.5 ± 3.6	0.0 ± 0.0
218 mg kg^{-1}	85.0	1	Top	183.6	0.0
218 mg kg^{-1}	85.0	1	Mid	27.1	0.0
218 mg kg^{-1}	85.0	1	Bottom	35.6	0.0
218 mg kg^{-1}	85.0	2	Top	238.7	1.1
218 mg kg^{-1}	85.0	2	Mid	95.5	0.0
218 mg kg^{-1}	85.0	2	Bottom	24.3	0.0
218 mg kg^{-1}	85.0	3	Top	251.9	0.0
218 mg kg^{-1}	85.0	3	Mid	38.8	0.0
218 mg kg^{-1}	85.0	3	Bottom	35.7	0.0
Mean-L ± SD	85.0 ± 12.0		Top	224.7 ± 36.2	0.4 ± 0.7
Mean-L ± SD	85.0 ± 12.0		Mid	53.8 ± 36.6	0.0 ± 0.0
Mean-L ± SD	85.0 ± 12.0		Bottom	31.8 ± 6.6	0.0 ± 0.0

Table A8. Extractable RDX and MNX contents in soil cores after 92 days of exposure to comp-B. Mean values and standard deviations are shown (N=3).

Comp-B Exposure	Initial RDX (mg core^{-1})	Block	Layer	Final RDX (mg core^{-1})	Final MNX (mg core^{-1})
Bare					
73 mg kg^{-1}		1	Top	167.8	0.0
73 mg kg^{-1}		1	Mid	393.2	0.0
73 mg kg^{-1}		1	Bottom	288.4	0.0
73 mg kg^{-1}	2293		Total	849.5	0.0
73 mg kg^{-1}		2	Top	476.4	0.000
73 mg kg^{-1}		2	Mid	124.3	0.000
73 mg kg^{-1}		2	Bottom	787.7	0.000
73 mg kg^{-1}	2293		Total	1388.5	0.0
73 mg kg^{-1}		3	Top	301.1	0.000
73 mg kg^{-1}		3	Mid	349.4	0.000
73 mg kg^{-1}		3	Bottom	831.5	0.000
73 mg kg^{-1}	2293		Total	1482.1	0.0
Mean-L ± SD	2293± 340		Top	315.1 ± 154.8	0.0 ± 0.0
Mean-L ± SD	2293± 340		Mid	289.0 ± 144.3	0.0 ± 0.0
Mean-L ± SD	2293± 340		Bottom	635.9 ± 301.7	0.0 ± 0.0
Mean-T ± SD	2293± 340		Total	1240.0 ± 341.0	0.0 ± 0.0
146 mg kg^{-1}		1	Top	493.456	0.000
146 mg kg^{-1}		1	Mid	79.879	0.000
146 mg kg^{-1}		1	Bottom	239.701	0.000
146 mg kg^{-1}	4944		Total	813.0	0.0
146 mg kg^{-1}		2	Top	1353.427	0.000
146 mg kg^{-1}		2	Mid	196.856	0.000
146 mg kg^{-1}		2	Bottom	389.044	0.000
146 mg kg^{-1}	4944		Total	1939.3	0.0
146 mg kg^{-1}		3	Top	1827.768	0.000
146 mg kg^{-1}		3	Mid	841.394	0.000
146 mg kg^{-1}		3	Bottom	942.925	0.000
146 mg kg^{-1}	4944		Total	3612.1	0.0
Mean-L ± SD	4944± 1187		Top	1224.9 ± 676.4	0.0 ± 0.0
Mean-L ± SD	4944± 1187		Mid	372.7 ± 410.1	0.0 ± 0.0

Comp-B Exposure	Initial RDX (mg core^{-1})	Block	Layer	Final RDX (mg core^{-1})	Final MNX (mg core^{-1})
Bare					
Mean-L ± SD	4944± 1187		Bottom	523.9 ± 370.5	0.0 ± 0.0
Mean-T ± SD	4944± 1187		Total	2121.5 ± 14108.4	0.0 ± 0.0
218 mg kg^{-1}		1	Top	868.7	0.0
218 mg kg^{-1}		1	Mid	525.6	0.0
218 mg kg^{-1}		1	Bottom	1959.2	0.0
218 mg kg^{-1}	6445	1	Total	3353.5	0.0
218 mg kg^{-1}		2	Top	2692.8	8.9
218 mg kg^{-1}		2	Mid	361.7	0.0
218 mg kg^{-1}		2	Bottom	599.0	0.0
218 mg kg^{-1}	6445	2	Total	3653.6	8.9
218 mg kg^{-1}		3	Top	2090.4	0.0
218 mg kg^{-1}		3	Mid	2354.2	10.9
218 mg kg^{-1}		3	Bottom	1356.6	0.0
218 mg kg^{-1}	6445	3	Total	5801.2	10.9
Mean-L ± SD	6445 ± 909		Top	1884.0 ± 929.4	3.0 ± 5.2
Mean-L ± SD	6445 ± 909		Mid	1080.5 ± 1106.1	3.6 ± 6.3
Mean-L ± SD	6445 ± 909		Bottom	1305.0 ± 681.5	0.0 ± 0.0
Mean-T ± SD	6445 ± 909		Total	4269.4 ± 1335.0	6.6 ± 5.8
***S. nutans* Vegetated**					
73 mg kg^{-1}		1	Top	861.8	0.0
73 mg kg^{-1}		1	Mid	58.7	0.0
73 mg kg^{-1}		1	Bottom	198.5	0.0
73 mg kg^{-1}	2293	1	Total	1118.9	0.0
73 mg kg^{-1}		2	Top	976.0	0.0
73 mg kg^{-1}		2	Mid	41.2	0.0
73 mg kg^{-1}		2	Bottom	72.0	0.0
73 mg kg^{-1}	2293	2	Total	1089.3	0.0
73 mg kg^{-1}		3	Top	708.2	16.8
73 mg kg^{-1}		3	Mid	60.5	1.6
73 mg kg^{-1}		3	Bottom	185.4	7.9
73 mg kg^{-1}	2293	3	Total	954.1	26.4

Comp-B Exposure	Initial RDX (mg core^{-1})	Block	Layer	Final RDX (mg core^{-1})	Final MNX (mg core^{-1})
S. nutans Vegetated					
Mean-L ± SD	2293± 340		Top	848.7 ± 134.4	5.6 ± 9.7
Mean-L ± SD	2293± 340		Mid	53.5 ± 10.7	0.5 ± 0.9
Mean-L ± SD	2293± 340		Bottom	152.0 ± 69.5	2.6 ± 4.6
Mean-T ± SD	2293± 340		Total	1054.1 ± 87.9	8.8 ± 15.2
146 mg kg^{-1}		1	Top	651.3	0.0
146 mg kg^{-1}		1	Mid	130.3	0.0
146 mg kg^{-1}		1	Bottom	842.3	0.0
146 mg kg^{-1}	4944	1	Total	1624.0	0.0
146 mg kg^{-1}		2	Top	799.7	1.3
146 mg kg^{-1}		2	Mid	1084.1	0.0
146 mg kg^{-1}		2	Bottom	496.0	0.0
146 mg kg^{-1}	4944	2	Total	2379.8	1.3
146 mg kg^{-1}		3	Top	1980.5	0.0
146 mg kg^{-1}		3	Mid	1233.8	0.0
146 mg kg^{-1}		3	Bottom	574.0	0.0
146 mg kg^{-1}	4944	3	Total	3788.3	0.0
Mean-L ± SD	4944± 1187		Top	1143.8 ± 728.3	0.4 ± 0.8
Mean-L ± SD	4944± 1187		Mid	816.1 ± 598.6	0
Mean-L ± SD	4944± 1187		Bottom	637.4 ± 181.7	0
Mean-T ± SD	4944± 1187		Total	2597.3 ± 1098.4	0.4 ± 0.8
218 mg kg^{-1}		1	Top	2286.1	0.0
218 mg kg^{-1}		1	Mid	337.6	0.0
218 mg kg^{-1}		1	Bottom	1812.1	0.0
218 mg kg^{-1}	6445	1	Total	4435.8	0.0
218 mg kg^{-1}		2	Top	2971.2	14.2
218 mg kg^{-1}		2	Mid	1188.8	0.0
218 mg kg^{-1}		2	Bottom	1235.7	0.0
218 mg kg^{-1}	6445	2	Total	5395.7	14.2
218 mg kg^{-1}		3	Top	3135.7	0.0
218 mg kg^{-1}		3	Mid	482.6	0.0

Comp-B Exposure	Initial RDX (mg core^{-1})	Block	Layer	Final RDX (mg core^{-1})	Final MNX (mg core^{-1})
S. nutans Vegetated					
218 mg kg^{-1}		3	Bottom	1814.7	0.0
218 mg kg^{-1}	6445	3	Total	5432.9	0.0
Mean-L ± SD	6445 ± 909		Top	2797.7 ± 450.6	4.7 ± 8.2
Mean-L ± SD	6445 ± 909		Mid	669.7 ± 455.4	0.0 ± 0.0
Mean-L ± SD	6445 ± 909		Bottom	1620.8 ± 333.5	0.0 ± 0.0
Mean-T ± SD	6445 ± 909		Total	5088.2 ± 565.2	4.7 ± 8.2

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
December 2009	Final report	

4. TITLE AND SUBTITLE

Phytoremediation of Composition-B Derived TNT and RDX in Herbaceous Plant-vegetated and Bare Lysimeters

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Elly P. H. Best, Jared C. Smith, and David B. Ringelberg

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

See reverse.

8. PERFORMING ORGANIZATION REPORT NUMBER

ERDC TR-09-10

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Corps of Engineers
Washington, DC 20314-1000

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release, distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This report describes a study in which phytoremediation of composition-B (comp-B) derived TNT and RDX was quantified in 0.5-m *S. nutans* (Indian grass)-vegetated organic matter and nutrient-poor soil over a 92-day period. The vegetation was allowed to establish in 0.5-m-high soil cores prior to amendment with ground comp-B mixed with the same soil, and effects and fate of comp-B derived TNT and RDX were followed in plants, soil, and leachate under greenhouse conditions. Remediation in vegetated soils exceeded that in bare soils for TNT up to a comp-B level of 218 mg kg^{-1}, and for RDX up to a comp-B level of 73 mg kg^{-1}. Thus, phytoremediation can be used as an effective remediation technology in a given range of explosives contamination. The greatest annual remediation potential was 58.5 g TNT m^{-2} and 42.4 g RDX m^{-2} in vegetated soils, and 54.5 g TNT m^{-2} and 51.0 g RDX m^{-2} in unvegetated soils. Remediation was attributed to a large degree to processes other than plant uptake, including bioremediation (plant-assisted or not), complexation with plant material and soil components leading to non-extractability, and photolysis (limited to the upper soil layer). Results of a comparison between ^{15}N-based and chemical-RDX-based mass balances, with ^{15}N derived from uniformly labeled ^{15}N-RDX, indicated greater incorporation of ^{15}N than of RDX in soil and plants of vegetated units than in soil of non-vegetated units. This was attributed to the incorporation of RDX metabolites generated by the increased microbial community biomass and activity, stimulated by exuded plant

(Continued)

15. SUBJECT TERMS

Composition-B	Grass	Soil
Explosives	Herbaceous plants	RDX
Forbs	Leachate	TNT
	Phytoremediation potential	

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED		100	

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) (Concluded)

U.S. Army Engineer Research and Development Center
Environmental Laboratory
3909 Halls Ferry Road
Vicksburg, MS 39180-6199;
SpecPro, Inc.
3532 Manor Drive
Vicksburg, MS 39180;
U.S. Army Engineer Research and Development Center
Cold Regions Research and Engineering Laboratory
72 Lyme Road
Hanover, NH 03755-1290

14. ABSTRACT (Concluded)

compounds, and to RDX transformation within the plants themselves. Sorption coefficients for Camp Shelby soil were low, indicating considerable potential for explosives leaching. These coefficients were three times greater for TNT than RDX. Despite the considerable leaching potential derived from the measured sorption coefficients, leaching was very low compared to loss of explosives due to processes other than plant uptake. The microbial communities in the upper soil layer showed decreased biomass with increasing comp-B exposures. Community shifts were subtle if at all. However, a presumed hysteresis effect was observed in the vegetated soil at a comp-B exposure of 146 mg kg^{-1} soil.